

Long haul ahead at Chernobyl

The temptation to blame Mr Mikhail Gorbachev for the muddle over the Soviet reactor should be suppressed; his predecessors, back to Lenin, are equally responsible.

THE continuing drama of the Soviet reactor accident at Chernobyl will drag on for several months to come. The past week's worry that the fuel of the damaged reactor would coagulate into a globule of material provided, by the decay heat of the fission products, with an energy source powerful enough to allow it to drill its way through the water table beneath the Ukraine, always a far-fetched notion, seems to have been put to rest by Hans Blix, the director-general of the International Atomic Energy Agency, now back in Vienna from Moscow. But there are plenty of other problems to cause anxiety in the months ahead. How seriously has the immediate environment been contaminated? What will happen to those living near the site who were compelled to sit around when they might reasonably have expected to be evacuated? How will the Soviet economy be set back by what is bound to be a serious interruption of its plans for generating electricity? What will be the consequences for nuclear energy programmes elsewhere? And what will be the political consequences of the Soviet Union's unwillingness, or perhaps just its inability, promptly to tell people elsewhere that something went disastrously wrong at Chernobyl on 26 April?

All these questions, general as well as particular, now hang on the provision of information from the Soviet Union. As the release of radioactivity from the reactor declines, people elsewhere will be less able than in the past two weeks to infer what is happening at the site. Even as things are, there is a huge range of uncertainty about the course of events. Taking Soviet statements at their face value, the accident began with a chemical explosion, while the reactor was operating at reduced or even zero power during a period of routine maintenance. But what kind of maintenance? Were the plant operators attempting to anneal the graphite? Or had some malfunction earlier come to light in the operation of this relatively new reactor? While efforts continue to deal with the emergency, the frenzy of which is easily imagined, and while the official inquiry is still incomplete, it is unreasonable to demand to know what went wrong. But afterwards, the Soviet Union will have an obligation to say publicly and internationally what went wrong.

But are not questions such as these purely domestic matters? No, partly because of the trouble and perhaps even the damage that has been done outside the Soviet Union, partly because there are other Soviet reactors of the same type in whose operation the rest of the world now has a legitimate and keen interest and partly because the Soviet Union has a responsibility by its membership of the international technical community to share what has been, to say the least of it, a salutary experience. Delay is understandable, but ultimately nothing less than full disclosure will suffice.

Scale

Exactly the same applies to the exposure of the neighbouring population of fallout from the damaged reactor. Soviet spokesmen and newspapers have been indignant in the past few days at what they consider malevolent speculations in the West about the scale of the disaster, and there are unhappily grounds for that complaint. But if it is the case that the first evacuation of people from the neighbourhood of the plant began only 36 hours

after the accident, and if the 30 km exclusion zone around the reactor was emptied only after a week, the Soviet Union must expect there to be general distaste for what on the face of things seems to have been a callous way of dealing with 50,000 loyal and compliant citizens. There is no reason to disbelieve what official Soviet statements have said, but they fall a long way short of satisfying legitimate curiosity. For example, it may be true that there were circumstances why local fallout was disproportionately smaller than that estimated from the quantities travelling beyond the Soviet borders, but even in that case there will be general (and legitimate) interest in what those special circumstances may be. Meanwhile, the Soviet Union should not be surprised if people elsewhere suppose that a substantial fraction of the now evacuated population has received a radiation dose that is significantly dangerous.

Exposure

What precisely may have happened to the Chernobyl population is complicated by the unavoidable complexities of radiation exposure. Few will have been exposed to such large acute doses of radiation that they are in danger of death by radiation sickness; that is the unhappy state of the 200 or so, presumably workers at the damaged reactor or elsewhere at Chernobyl, who were taken to hospital after the accident. But the general dose rates quoted by the Soviet authorities as a few roentgens an hour immediately after the accident would imply that a substantial proportion of this general population will have accumulated in the first day a lifetime's permissible exposure to radiation. (The international standard is that no worker should suffer more than 3 rem in a year, with a general understanding that such exposure should not arise year in and year out.) The result, among such a large number of people, will almost certainly be an increased cancer load some years from now, not to mention the genetic defects that would afflict the next generation. There will be a temptation, in these forbidding circumstances, for the Soviet authorities to stick their heads firmly in the sand, not going out of their way to keep track of those exposed. That would be to make things worse. It is bad enough not to have evacuated the population sooner, but that is just barely explicable as muddle. Now that there is time available, it would be inexcusable not to follow the cases of those exposed at Chernobyl.

None of this implies that no attention should be paid to Moscow's excuse for acting so slowly after the accident. It is, unfortunately, all too easy to accept that the managers of the reactor plant at Chernobyl would at first have taken it on themselves to put out the fire that apparently followed the explosion, and how the regional authorities would have kept the news from the centre at Moscow until it had become plain that the accident was too big to be contained. Although people in the West have now stopped scoffing at the Soviet Union for this tragic accident, the temptation now is to complain that the handling of the accident squares badly with Mr Mikhail Gorbachev's declared objective of making the Soviet Union a more open society. But this jibe rests on a gross misunderstanding of Soviet society, and of its most serious internal weakness.

Public administration is shared between centralized organiz-



ations, of which the Commission for the Utilization of Atomic Energy is only one, which are autonomous on a day-to-day basis and which can be influenced from outside only occasionally, at a Party Congress, for example. This is why public administration is so tolerant of its own mistakes, and why even the most energetic new brooms cannot expect to have much influence quickly. This system, the source of the muddle and bureaucracy that shackles the Soviet Union, is ironically the instrument of the dictatorship of the proletariat. The means of production, from agriculture to atomic energy, are centrally owned and operated, supposedly on behalf of the proletariat, but by organizations that seem to have scant regard for people's interests. There is nothing new in this arrangement. The central organizations, inflexible since Lenin's time, are in many ways but extrapolations of the czarist bureaucracies. Mr Gorbachev's wish that the system would become more open will not be met until these quasi-autonomous inadequately accountable organizations are cut down to size. The obvious failures of the system in the past two weeks should give him an excuse to tackle its weaknesses, from which many good things may flow, a greater freedom for Soviet science in particular.

The often-prurient interest by the West in events at Chernobyl stems from the sense that what happened there may strike deeply at the way in which Soviet life is organized. The comparison with the disaster to the US shuttle in January is bound to spring to mind. That was as big a blow to national pride as anything that has happened in the Soviet Union; ironically, as it turns out, there are now complaints in the United States (by the chairman of the investigating commission in secret testimony to the US Congress) of a cover-up. There may be, most probably is, a lot wrong with US society, yet nobody has yet suggested that there is no way forward except by radical change. Mr Gorbachev's Union of Soviet Socialist Republics is not so lucky. □

Life without Joseph

The British government is to be modestly reshuffled, in which process lie opportunities.

Sir Keith Joseph, for long one of the most controversial members of the British government, will no doubt be astonished by the way in which his public reputation is changing. Sir Keith let it be known some weeks ago that he would not be seeking re-election to the House of Commons at the next general election, which must be held during the next two years, whereupon the government let it be known that he would probably vacate his post as Secretary of State for Education and Science before the end of the present Parliament. That is a curious happening in itself; there is no obvious reason why a minister who will not be returning after the election should not, in the meantime, soldier on, which has led to the obvious speculation that Sir Keith's colleagues have come to share at least the mildest version of the common criticism of him, that he is full of good ideas at whose execution he is inept.

Now, after the spectacular loss of a by-election last week and a poor showing in the local elections, the word is getting about that Sir Keith's departure from his post (but not necessarily from the government) will be sooner rather than later, not this week (which would seem over-hasty) nor even next (which would still seem a little soon), but perhaps the week after that. At the same time, there are suggestions that the British government will use the occasion for a wider shuffling of responsibilities. The remarkable consequence, for Sir Keith, is that the nostalgia that appears to accompany the departure of politicians from high office, impelling their loudest critics to declare that they were statesmen after all, has engulfed Sir Keith even before he has cleared his desk. After months of bruising argument with teachers in British schools, for example, there are now many educationalists who will acknowledge that he has been right to ask for mechanisms for improving the quality of teaching. Do

Sir Keith's constituents fear losing the man they love to hate?

Part of the British government's calculation seems to be that education is not the tediously boring matter it has seemed for the past several years, but instead an issue by means of which electoral unpopularity might be exorcized. The calculation is surely correct; great credit would certainly accrue to a government that was able to offer schooling of high quality to the whole of the population, and to provide higher education on a scale and of a character that would ensure rising or even merely continuing prosperity. But the goal may not be as easily attainable as the optimists among the government's strategists suppose. School systems and universities are sociologically complicated institutions which cannot be changed, or made to grow, at the snap of a politician's fingers (as the attempts at contraction over the past few years have shown). At the school level, hopes for a better system may in the present circumstances and perhaps permanently be frustrated by the difficulty of recruiting talented people who are able and willing to teach in schools. But the British government is also uncertain about its ambitions for the transformation of secondary education. For several years, in parallel with the general decline of morale of secondary school staff, it has been encouraging a series of experiments in vocational education under the aegis of the Department of Employment; now there is talk of putting the traditional and new initiatives together in a single ministry (which is a sensible idea). But the government would be mistaken if it thought that such a decision would magically bring about the general improvement that it rightly seeks. The best it can hope for quickly is that there might be a prospectus for improvement so persuasive, and so generously backed with promises of funds, that electors would suspend what otherwise is likely to be a harsh judgement.

In higher education, there is a different set of problems to be tackled. One sensible and simple step would be to give up the pretence that the demand for higher education is bound to fall, so that the provision of higher education must be forced to contract. Why not just leave the imponderable question to the market, avoiding in the process the contumely that must fall on governments that actively close institutions? Another issue is that of research, which is anomalously administered by Sir Keith's huge ministry, largely preoccupied with schools (and teachers' pay). If there is now to be a reorganization, why not take the opportunity to put civil research somewhere else, perhaps (as the House of Lords argued five years ago) with a part-time minister as its titular head? Moreover, the present is a splendid time for making such a change, for there is likely to be at least one under-employed member of the reorganized cabinet, Sir Keith Joseph himself, who has at least learned enough in the past few years of the ambitions of the research enterprise to have become a sympathizer if not a staunch defender.

Nobody can tell whether the government will have the stomach or the inclination for this sensible decision. For several decades, it has been plain that Britain, now almost proud of the way in which other people trade on its bright ideas, would be a more prosperous place if better use were made of science. From time to time (most recently, in the latest report of the government's favourite advisory committee, the Advisory Committee for Applied Research and Development just two weeks ago) people dare to say this publicly. Yet nothing happens, or nothing very much. Part of the trouble, when a government's calculation of electoral advantage has been foreshortened by political events, is that research is not numerically a substantial constituency. But even then, the sensible course might be well worth following. For even if research has become merely a financial burden, it remains a nuisance. Its practitioners are always writing to the newspapers complaining about their treatment. Would it not be a clever wheeze to get research off the government's back for the time being by giving the research community the rope with which to hang itself on the proposition that it knows as well as cabinet ministers how Britain might be made prosperous again? □

AIDS

US plan for foundation in trouble with French

Washington

THE United States has announced its intention to create an international foundation to promote research into acquired immune deficiency syndrome (AIDS). Health and Human Services Secretary Otis Bowen made the announcement in a speech to the 39th World Health Assembly last week in Geneva, Switzerland.

Bowen's speech was delivered just days after an apparent breakdown in negotiations between the Pasteur Institute and the US Department of Health and Human Services (HHS) to resolve the legal dispute over the patent of the blood test for AIDS. A lawyer for the Pasteur in Washington claimed that a similar plan for an international foundation had been suggested by HHS lawyers as a basis for settling the dispute.

In a press release issued last week before Bowen's speech, Pasteur Institute director Raymond Dedonder claimed that the US government was moving toward awarding the blood test patent to the Pasteur Institute, and accused HHS of "making a public relations gesture rather than facing the reality of the situation". On 29 April, the US Patent and Trademark Office agreed to investigate whether the Pasteur patent should take precedence over the patent already awarded to Robert Gallo and his colleagues at the US National Institutes of Health (NIH) for the AIDS blood test (see *Nature* 321, 102; 1986).

A spokesman for HHS maintained that negotiations were still going on with the Pasteur to settle the patent issue. But according to a lawyer for the Pasteur Institute, HHS informed Pasteur's lawyers on 2 May that if they did not agree to go along with the international foundation, HHS would proceed unilaterally with the idea.

As originally formulated, the proposed foundation would support basic research on retroviruses, with special emphasis on the virus causing AIDS. A nine-member board would select research projects for funding. The board would consist of three members each from the United States and France and three additional members from other countries chosen by the initial six. Acting Assistant Secretary of HHS Donald Macdonald said in an interview that there were still a lot of "loose ends to be tucked in", but he confirmed that potential board members have been contacted and papers of incorporation have been drawn up. Macdonald said he hoped that a final decision to go ahead with the foundation could come by the middle of June.

But Macdonald explicitly denied the suggestion that the foundation proposed by Bowen was linked to settling the patent dispute. He claimed it was a "unilateral action to encourage international cooperation", and would not require French agreement.

Caroline Chaine, speaking for the Pasteur Institute, said the institute could not go along with the foundation idea because it would "take monies to which we have

rights". She described the proposal as "unacceptable" for financial, legal and "ethical" reasons — because the Pasteur had after all made the discovery of the AIDS virus and offered the compromise of sharing royalties.

The stakes in the patent dispute are high. In addition to the prestige of the researchers and institutions involved, the US Department of Commerce estimates that the patent currently held by the United States is generating revenue at the rate of \$2 million per year. That figure could grow substantially if blood tests become more widely used. While \$2 million represents a small fraction of NIH's current budget for AIDS research, it is approximately two-thirds of the Pasteur's 1985 AIDS budget.

Joseph Palca

French science

Research gives place to projects

THE powerful new French ministry of finance is dictating terms to the ministry of research, it emerged last week. In the process, the ministry seems to be going far beyond its traditional role into the detailed examination of research policy and perhaps even beyond its competence.

The case in point is the budget of the principal body supporting basic science in France, the Centre National de la Recherche Scientifique (CNRS), which has fallen by 10 per cent in the cuts recently imposed on research by the new government. CNRS would normally have expected to make its own decisions about the allocation of the cuts, but the hard choices made by its council last week have had to be referred back to the ministry.

CNRS at its meeting last week decided that it would protect its laboratories' working budgets for materials and equipment. The council therefore elected to produce the bulk of the FF230 million (£23 million) savings forced on it by the government in the form of 25 per cent cuts in special programmes and 20 per cent in spending on equipment and buildings. This left only 7.5 per cent (FF80–90 million) to be shaved off research budgets.

It seems, however, that the finance ministry has said no, and demanded a cut of at least 10 per cent in the laboratory budgets, which would mean savings of another FF30 million. This would mean, in turn, that less would have to be removed from the special programme or building budget, but that seems hardly to be the real issue, which is whether it is CNRS and the ministry of research and higher education that are controlling research policy, or the ministry of finance.

Meanwhile, scientific hackles are also being raised in some quarters at the preferential treatment being meted out to some of France's big technological programmes, notably space development,

which is at present sustained by the success of the Ariane space launcher, fully-booked far into the future, with the SPOT Earth-observation satellite now fully operational and with plans for Hermes, a European mini-shuttle 50 per cent financed by France, looking stronger after West Germany last week indicated its likely commitment to the project.

Hermes, less ambitious than the US space shuttle, is likely to cost some FF15,000 million (£1,500 million). That, however, is money yet to be spent, and details of the 1986 spending of the Centre National d'Études Spatiales (CNES), the French national space agency, published last week, already demonstrate dramatic increases agreed by the previous socialist government and which have not been touched by the new conservative government.

The 1986 CNES budget is up more than 25 per cent compared with 1985, with most of that increase from public funds, to reach a figure of FF6,042 million this year. By far the greatest part of this comes in the form of direct technological programmes, including SPOT, telecommunications and direct broadcast satellites, together with the development of advanced versions of Ariane. Scientific experiments *per se*, many of them in cooperation with the Soviet Union, will cost CNES just FF185 million, only 3 per cent of its total budget this year.

However, this concentration of technological "great programmes" (such as nuclear power, the European Airbus, the high speed train, and space) is fully within the French tradition and even continued under the previous socialist government. Under the new, right-wing government, the tradition largely in the hands of the élite French engineers is emerging unscathed, while the basic science is suffering.

Robert Walgate

Chernobyl

Intangible fallout from reactor

OFFICIALS of the International Atomic Energy Agency at Vienna were confident, earlier this week, that they would be able to convene a conference on the damaged Soviet reactor at some stage during the summer. The proposal was raised by the director-general of the agency, Hans Blix, on his visit last week to Moscow. Although a date has not yet been fixed, the Soviet authorities have apparently indicated their willingness to attend, and to bring whatever data are available to them.

The Blix visit was apparently a great success, according to sources close to the agency. Apart from establishing with the Soviet authorities channels of communication for the weeks ahead, the agency officials have been able to check the meaning of statements appearing in routine announcements from Moscow.

Data made public by Blix at press conferences in Moscow are said to be reliable as far as they go, but they refer chiefly to the zone around the plant and not to that within the perimeter fence. But it appears that during the course of a helicopter journey over the damaged plant on 8 May, the personal dosimeters worn by Blix and his companions registered 350 mrem an hour at a height of 400 m above the plant and at a distance of 800 m. This isolated measurement suggests that the neighbourhood of the plant was still then heavily contaminated.

In the 30-km evacuation zone around the plant, the Vienna agency endorses Soviet statements that dose rates have fallen steadily. The maximum dose rate recorded in the nearby township is said to have been between 10 and 15 mrem an hour soon after the accident, to have fallen to between 2 and 3 mrem an hour on 5 May and now to have declined to 0.15 mrem an hour at the perimeter of the zone.

Conditions at the plant itself are far from clear. Reports that civil engineering works were in hand beneath the reactor suggested to some in the West that those on the spot, having buried the reactor core in 4,000 tonnes of sand and clay, mixed with boron and lead, were concerned that this insulating blanket would allow the presumably molten core to eat its way through the concrete foundations of the reactor. Although the thermal power of freshly irradiated light-water reactor fuel decreases rapidly (by a factor of 50 in 15 minutes) after the shutting down of a reactor, heat production by fission product decay within the fuel remains at close to 0.5 per cent of normal operating power for several days thereafter. This means that the blanked-off reactor core at Chernobyl will have been producing more than 10 MW of thermal power even two weeks after the accident.

In the event, it appears that the threatened catastrophic meltdown has not materialized, although the Soviet authorities say that their objective has been to isolate the reactor from its surroundings as far as possible. (Levees separating the site from the nearby Pripyat river have been raised to prevent run-off from the environs of the plant.)

The first full account of the disaster within the Soviet Union was provided by *Pravda* on 6 May, in the course of a long article more concerned with the heroism of those who had helped to fight the fires than with factual statements.

Further details were given later on the same day at a press conference attended by Deputy Premier Shcherbina, Dr Andronik Petrosyants (chairman of the state committee on the peaceful uses of atomic energy), Vorob'ev, the deputy minister of health, and the director of the institute of power engineering, Emelyanov.

There is particular satisfaction outside the Soviet Union that the members of the

disaster commission include Academician Velikov, the independently-minded vice-president of the Soviet Academy, who is said by the Soviet press to have been the chief source of technical opinion.

Although the several European states bordering or near the Soviet Union have reported increased levels of activity during the past two weeks, none has suggested that the effects will be permanent or damaging. But in one of the most level-headed of the statements put out by European governments, the Polish government explained (early last week) that it had placed restrictions on the supply of milk to children because radio-iodine concentrations in milk had exceeded 1,000 becquerels per litre in some parts of north-east Poland, which is the limit suggested by the International Agency for milk fed to children in an emergency.

Complaints that the Soviet Union has been niggardly with information about the disaster have been met both with denials and with the statement that Moscow was inadequately informed by Kiev of the potential scale of the disaster at Chernobyl. The general belief that heads would roll appears to be being fulfilled. □

Tokyo summit wants to know more

Tokyo

LEADERS of the seven leading industrialized democracies arriving in Tokyo for the annual summit last weekend quickly found themselves with an extra topic on the agenda: the Chernobyl nuclear accident. A Japanese call for a response to the incident was backed by the British on Sunday and by Monday a joint statement had been issued.

The statement was essentially a call for the Soviet Union to provide information about the accident and to urge the "early elaboration of an international convention" to commit countries using nuclear power "to report and exchange information in the event of nuclear emergencies or accidents". But the opportunity was not missed to point out the "exacting standards" of "design, manufacture, operation and maintenance" of the nuclear facilities of the seven summit nations (the United States, the United Kingdom, France, West Germany, Italy, Canada and Japan) and of their acceptance of the responsibility to provide "prompt, detailed and complete information on nuclear emergencies and accidents".

The Chernobyl incident also provides a clear message for arms control, according to British Prime Minister Margaret Thatcher, speaking at the end of the summit. She emphasized that "security at a lower level of arms" required verification. If information had been so difficult to obtain from the Soviet Union about a civil reactor, then there would be much greater difficulties in the military field.

The summit statement on Chernobyl coincided with the return of Japanese tourists who had been in the Soviet Union at the time of the accident and the first detection of radioactive fallout in the Japanese skies. For one tourist brought back from Kiev the incident was a tragic reminder: he was a 60-year-old survivor of the Hiroshima atomic bombing. Radiation 35–140 times the natural average was detected from tourists' luggage and some was placed in custody by the government. Over Japan, radioactive fallout was first detected by high-flying F4 fighter jets on 4 May and by 11 May had been detected in rain in more than half of Japan's prefectures and as far south as Okinawa.

The accident has done little to shake Japan's nuclear industry and a wave of selling of the big electric power utilities' issues on the Tokyo stock market was short-lived. The government showed little reaction to calls by the Japan Socialist Party and the Japan Communist Party to halt the construction of new nuclear plants and to re-examine radically nuclear power policy. Thirty-two nuclear power plants are in operation in Japan, with permission already granted for the construction of another 18 and a further dozen being planned. The accident is bound, however, to strengthen the anti-nuclear movement centred on Fukui prefecture where eleven nuclear plants have been built in one small region. Local citizens have just begun court proceedings to try to stop the construction of the first fast-breeder reactor *Monju*. **Alun Anderson**

US analysis incomplete

Washington

EFFORTS in the United States to estimate the long-term consequences of the Chernobyl reactor accident were hampered last week by the lack of detailed information on the radionuclides released. But researchers at Lawrence Livermore (LL) National Laboratory now believe that most of an assumed core inventory of 80 million curies of iodine-131 and 6 million curies of caesium-137 was released during the disaster, a "mammoth emission" in the words of Joseph Knox, head of LL's atmospheric release advisory centre. The amount of iodine-131 released during the US Three Mile Island reactor accident was, by comparison, a mere 15 curies.

A substantial proportion, perhaps as much as half of the total emitted, appears to have been borne with the initial explosion to a height of 20,000 feet or more and then transported eastwards, according to Knox.

The other half of the activity release was injected at low altitude and was initially blown north-west, according to Knox. One of the highest atmospheric activities recorded outside the Eastern bloc was in Stockholm, where a level of 5,130 picocuries of iodine-131 per cubic metre of air was found.

Monitoring data are being provided by many friendly and neutral countries and by US embassies around the world, as well as by the domestic monitoring network. The inventory of nuclides appears to be much as would be expected from a civilian power reactor: besides iodine and caesium, tellurium, ruthenium and some technetium are found. But the US inter-agency task force collating data on the accident is puzzled by an almost total absence of the expected strontium-90.

Knox's group, with its normal staff of 22 supplemented because of the Chernobyl work, has been working more than 12 hours per day since the accident to model the atmospheric consequences of the release with supercomputers.

Meteorological data for the simulation are being supplied by the US Air Force's Global Weather Center. Preliminary simulations suggest that adult thyroid doses during the two days following the accident would have surpassed 1 rem over an 85,000 km² swathe of the Soviet Union, extending into northeastern Poland.

It will be almost impossible to get a clear picture of the much higher radiation doses and expected prompt radiation deaths close to the plant without Soviet data on activity levels and evacuation timetables.

There is equal uncertainty about the circumstances that caused the accident. The International Atomic Energy Agency (IAEA) team visiting the area has reported that the reactor was operating at

low power for scheduled maintenance when the accident occurred.

Frank Clikeman, professor of nuclear engineering at the University of Indiana, observes that the RBMK-1000 reactor is likely to have a positive power coefficient in a loss of coolant accident, meaning that the rate of power generation in the reactor would increase as its temperature went up; this is because the graphite moderator, unlike water, does not expand significantly as it is heated. Attempts to rescue the situation may have been hindered by a loss of electrical power.

Despite earlier fears, the possibility of a complete meltdown of the reactor fuel and its sinking through the floor of the reactor to contaminate ground water seemed to be receding late last week.

The nuclear power industry in the United States is stressing that plant design safety standards in the United States are much higher than in the Soviet Union; in particular, the Soviet reactor had no containment building. But it is nevertheless inevitable that heightened concern over nuclear safety will delay licensing of the 20

or more nuclear plants expecting approval in the next few years.

Congress has already postponed action on renewal of the Price Anderson Nuclear Insurance Act and procedures for licensing standardized reactor designs pending a fuller evaluation of the Chernobyl accident. The Secretary of Energy, John Herrington, has ordered a special safety review of the only US reactor with appreciable similarities to Chernobyl, the "N reactor" at Richland, Washington state, which produces nuclear material for bombs. This reactor is also graphite-moderated and has no containment building.

There are other US reactors that lack containment buildings at the Department of Energy's Savannah River site in South Carolina, also a weapons site.

Some US scientists have made informal proposals for collaborative studies with overseas scientists to Professor Walter Rosenblith, foreign secretary of the US National Academy of Sciences, but as yet the academy (which recently signed a scientific collaboration agreement with the Soviet Union) has made no formal proposals.

Tim Beardsley

Where does the blame lie?

THREE Ukrainian party officials have been disciplined in connection with the Chernobyl disaster. According to the Soviet newspaper *Pravda* on 12 May, one was expelled from the party for indifference and shirking his duty to look after refugees, a second was severely reprimanded for the same offences, and the third was reprimanded for failing to give "timely and accurate" information on the disaster.

The dismissals came after several days of veiled admissions by Soviet officials that there had been slackness and confusion in the disaster area. Thus eulogies of the emergency teams fighting the reactor fire and building defences beneath it in case of meltdown were coupled with criticism of the few who had failed to show the appropriate heroism and steadfastness in what was described as the "front line".

Western critics have attacked the Soviet treatment of the disaster for the failure to provide sufficient information both to their own population and to other countries liable to be affected by the radioactive cloud and for their failure to evacuate the local population in good time. A careful reading of the Soviet press over the past week suggests that, between 2 and 7 May, Moscow gradually became aware that the Western allegations, which it had denied in all good faith, had, in fact, some substance.

Evacuation had indeed been delayed at Chernobyl itself for a whole week and the radiation hazard was far greater than announced. On 8 May, the Soviet media gave accounts of long queues for any available train out of the city. *Pravda* even

called on people not to panic.

According to this reading of the situation, for about 10 days after the accident, officials in Moscow were desperately trying to tell the world the truth as they knew it, but all the time were inadvertently relaying misinformation. The blame for this has been fixed on the officials on the spot, with possible further reprimands and dismissals gradually making their way up the Ukrainian party and state hierarchy. The Ukrainian and Byelorussian republics have, formally speaking, the greatest degree of autonomy of all the constituent republics of the Soviet Union.

Although the Soviet power industry, and the nuclear programme in particular, are organized on an All-Union basis, responsibility at Chernobyl, at least for the post-disaster chaos, has been laid by Moscow at the door of the Ukrainians.

But Moscow cannot eschew all responsibility. The original claim was that 50,000 people had been evacuated from the vicinity of the power station on the afternoon of Sunday, 27 April. Even if plans for evacuation had been made when the first signs of trouble developed on Friday, 25 April, the logistics of the operation must have made the claim somewhat improbable. Yet nobody seems to have thought to verify the claim. If, as is now widely believed, it was the visit of Prime Minister Nikolai Ryzhkov to the area at the beginning of May that revealed to the central authorities that the town of Chernobyl had not been evacuated, it must have been Moscow, and not Kiev, that suppressed this fact for a further five days. **Vera Rich**

SDI snubs APS

Washington

THE Strategic Defense Initiative Organization (SDIO) delivered a public snub to the American Physical Society (APS) recently by boycotting at the last minute a symposium on strategic defence at APS's spring meeting in Washington. According to Richard Bleach, one of two invited speakers from SDIO who failed to turn up at the meeting, SDIO senior management decided that "some of the abstracts for the session were so political that we should find some other place to present [our results]".

Bleach, who works in the SDIO programme integration office, had been due to represent SDIO together with James Ionson, head of SDIO's innovative science and technology office. The title for the session was "Impacts of the Strategic Defense Initiative on the physics community". In addition to speaking at the symposium, Bleach and Ionson had been due to appear at an APS press conference.

Ionson let APS know in advance that he would not attend the press conference, but, according to Robert Park of APS, no word was received that he also would not appear at the symposium. Ionson says it would be improper for him to appear at someone else's press conference without approval from SDIO management, which was apparently not sought; he says he asked his office to let APS in New York know that he would not attend.

Bleach told APS he would not attend the symposium or the press conference on the day they were to occur. Bleach says he was told by senior SDIO management not to attend the session after they read published abstracts of other talks to be given at the symposium: one, by Charles Schwartz of the University of California at Berkeley, states: "The strategic defense initiative... will push strategic instabilities so far that it will be difficult, in a time of severe crisis, for national leaders to avoid the option of pre-emptive attack". Schwartz goes on to say that "this is the career awaiting the major portion of our physics students" and proposed to discuss "several forms of resistance".

SDIO has other reasons to be irritated with APS besides the allegedly "political" meeting. The society has recently given much publicity to an opinion poll of its members which found almost a two-to-one majority believing SDI was bad for national security.

Anti-SDI feeling was certainly prevalent at the Washington meeting: an official desk was promoting anti-SDI literature. At the ill-fated press conference, representatives of *Fusion* magazine, a publication linked to political maverick Lyndon LaRouche, castigated APS for not giving similar space to non-governmental supporters of the Strategic Defense Initiative.

Tim Beardsley

US in space

Delta failure a big set-back

Washington

THE spate of calamities that has struck the US space programme this year has led the National Aeronautics and Space Administration (NASA) into difficulties both with the Office of Management and Budget and with Congress. Damaging revelations continue to surface about slipshod safety procedures that may have contributed to the explosion on 28 January of the space shuttle Challenger and, most recently, the failure on 3 May of an unmanned Delta rocket at Cape Canaveral.



Meanwhile, a proposal to provide NASA with funds for a replacement shuttle orbiter remains stalled in the White House.

The accident on 3 May, in which a Delta launcher and a National Oceanic and Atmospheric Administration weather satellite (GOES-7) were destroyed following a rocket engine failure, means that the United States now has no reliable means of launching payloads of over 2,000 lb into orbit, at least until the causes of the recent accidents have been determined and corrective measures taken.

NASA had planned to launch three more Deltas for paying customers (one more weather satellite and two Strategic Defense Initiative experiments) before turning the rocket over to the private sector; the accident will only strengthen the resolve of the US Air Force to expand its independent launch capability, and distance itself from the administration's previous policy of relying exclusively on the shuttle for military access to space.

There are some 17 Atlas rockets of different types available for military use, most already assigned to a launch. But because of similarities between the Delta engine and that of the Atlas rocket, Atlases are also grounded until the Delta problem is tracked down.

Meanwhile, the Air Force has its own problems, with the explosion (also last month) of a Titan 34D rocket seconds after lifting off from Vandenberg Air Force base in California. In response to

the shuttle loss and the Titan accident, the Air Force is to procure 13 additional Complementary Expendable Launch Vehicles (CELVs), otherwise the Titan 34D7. These will be extra to the 13 Titan-2s being refurbished for Air Force use.

NASA has still not prepared a detailed supplementary budget request for fiscal year 1987 in the light of the Challenger and other accidents, which is becoming the source of some frustration on Capitol Hill. NASA officials say they are convinced of the need for a new orbiter, but a decision on who should pay for it apparently still not been settled, with the Office of Management and Budget opposing the use of extra funds.

The total cost of a new orbiter, modifying the suspect solid booster joint on the other orbiters and the replacement of spare parts, comes close to \$3,000 million, so if NASA had to find the cost of a new orbiter out of its own budget it would be able to do very little else for a year.

Part of the argument now hinges on whether the cost of a new shuttle could be justified as a national security emergency; if it could be, it need not be included in the deficit reduction target of the Gramm-Rudman law. An administration decision is expected next month.

A further difficulty is that NASA has not yet published a proposed plan for dealing with the backlog of shuttle launches once they do begin again, which is now thought unlikely before July 1987. As about 16 launches per year had been planned, the backlog is likely to be around 24. Given that military payloads will have priority over others, it seems clear that scientific missions will be greatly delayed. One suggestion is that the Galileo mission to Jupiter would take a "delta vega" trajectory via the inner planets, which would take two years longer than the original trajectory, but would eliminate the narrow launch window that puts Galileo in competition for a slot with Ulysses, the joint mission with West Germany to the poles of the Sun.

There remain several important safety hurdles to be negotiated. The Centaur upper stage used in these missions would be the first liquid-fuelled upper stage to fly in the shuttle, and special safety reviews of the issue have been instituted. The intended use in the spacecraft of radioactive thermal generators has only added to nervousness about the consequences of another shuttle accident.

Commercial satellite launches have also been held up by the shuttle accident, and Arianespace, the competing French carrier, has taken some extra bookings. The order books are now full until 1989, and Arianespace says it cannot greatly in-

crease its launch rate. China is eager to fill the breach, and according to *China Daily*, the two satellites retrieved from faulty orbits by the shuttle will be the first commercial launches in China's Long-March-3 rocket. The launches are scheduled for 1987.

Meanwhile NASA officials have to face the music over the sudden change in the agency's fortunes. NASA's director for space flight, Rear Admiral Richard Truly, was taken to task last week by Senator Albert Gore (Democrat, Tennessee) over reductions in the number of NASA reliability and quality assurance personnel. According to Gore (relying on documents leaked by a NASA employee), these averaged 71 per cent across the agency over 15 years, as compared with a 30 per cent reduction in the size of the agency as a whole. Gore concluded that NASA, which was already drifting, "is now drifting further".

Tim Beardsley

Tokyo summit

Nakasone disappointed

Tokyo

OTHER than the Chernobyl incident, little attention was given to science and technology issues at the summit although appreciation was officially recorded for the continuing efforts at the international series of "biology and ethics" meetings instigated by Prime Minister Yasuhiro Nakasone (see *Nature* 308, 395; 1984). That discussion of ethics has been the sole outcome of the numerous international technology research projects put forward at successive summits is some indication of the difficulty of international cooperation outside basic science.

Conspicuous by its absence at this year's summit was the "Human Frontier Project". Thought up by Japan's Ministry of International Trade and Industry (MITI), the project for large-scale research in biology (*Nature* 320, 296; 1986) with application in mind was not presented at the summit as had been hoped. Officials from MITI have toured major Western nations to explain the project, but lack of clear details and an assured budget have so far elicited only polite encouragement. Whether anything more will come of it now depends on whether Japan's Ministry of Finance can be persuaded to put up funds on a scale sufficient to support research abroad as well as in Japan, an entirely new idea to the ministry.

During the summit, Nakasone's mind may well have been on a quite different kind of technological development — the increasingly sophisticated mortars developed by the extreme left-wing organization *Chukakuha*. Repeated rumours that the *Chukakuha* had developed mortars with a range of up to 4 km.

As it was, the rumours turned out to be

Tropical diseases

More research, more money?

A LEANER management and a fatter programme of basic and clinical research are planned for the Special Programme for Research and Training in Tropical Diseases (TDR) by its new director, Dr Tore Godal. When he takes over next month, initially for two years, from Dr Adetokunbo Lucas, Godal does so with the prospect of an increasing budget for TDR, which is financed by the United Nations Development Programme, the World Bank, the World Health Organization and individual countries.

Chief among the donating countries are the United States, Sweden, Denmark and Norway, from which Godal comes. The Scandinavians have always been generous towards TDR, says Godal, and there is a good chance that they will become even

more so. He would also like to persuade the United States and Britain to increase their contributions. Both take more money from TDR than they contribute, although there the similarity ends; at \$2.0 million, the US contribution was 10 per cent of the total annual budget, larger than that of any other country, whereas the UK contribution, which surpassed \$1.3 million in 1979, was cut to zero in 1982 and was \$0.3 million in 1985.

Godal would like TDR to spend more on basic aspects of parasite biology, preparing the way for new approaches to chemotherapy and vaccination, and on field research into TDR's target diseases, malaria, schistosomiasis, filariasis, leprosy, leishmaniasis and trypanosomiasis. If no more money is forthcoming, this will have to be at the expense of clinical research and the blind screening of candidate drugs. For the time being, TDR's income in terms of US dollars is increasing as a result of the fall in the value of the dollar against the currencies of many donor countries.

Malaria remains the top priority of TDR's diseases, particularly with the growing problem of resistance of malaria parasites to drugs, whereas priorities among the other five diseases will continue to change with circumstances. In recent years and with growing signs of success, TDR has spent an increasing portion of its budget on research into leprosy, the subject of Godal's own research in Oslo and with Ethiopian collaborators.

Godal views with some anxiety a proposal, to be considered by the board of TDR in June, to allow donor countries to earmark a proportion of their contribution for research on particular diseases. If implemented, this could mean that it was not scientific criteria alone that would determine what was funded, and it is the strength of its science that has accounted for TDR's past success in Godal's view.

He is less anxious about the problem of relationships with industry. While there is often a conflict of interest between donors and the pharmaceutical companies that are needed for the large-scale production of any products of TDR research, Godal considers that some of these problems have been smoothed out by experience and that *ad hoc* solutions will be possible.

With regard to administration and management, Godal's plan is for simplification. Application will be less hierarchical, and less staff will be needed.

Godal will continue to look after his doctoral students in Oslo but will otherwise devote himself wholly to TDR. He starts in Geneva on 9 June; Lucas leaves on 1 July to start working for the Carnegie Institute in New York.

Peter Newmark

Alun Anderson

Vaccine engineering

Violation of rules alleged

Washington

A FIELD test of a new vaccine that went relatively unnoticed when it occurred two years ago has recently been the subject of several investigations after doubts surfaced about the steps taken by the US Department of Agriculture (USDA) in approving the vaccine for sale. The latest report comes from the Baylor College of Medicine Recombinant DNA Subcommittee criticizing faculty member Saul Kit for participating in a test of his pseudorabies vaccine that "violated" National Institutes of Health (NIH) Recombinant DNA Guidelines and Baylor College of Medicine policies.

The Baylor report comes hard on the heels of a letter sent to the NIH Recombinant DNA Committee (RAC) by the Texas A & M University Institutional Biosafety Committee complaining that Kit's experiments constitute a violation of NIH guidelines. Former Texas A & M faculty member Stewart McConnell collaborated with Kit on the field experiments.

The test in question involved inoculating approximately 1,400 pigs on the Maddox farm in west-central Texas where an outbreak of pseudorabies occurred in June 1984. Pseudorabies is caused by a herpes virus, and can be fatal to young pigs.

Since 1981, Kit has regularly sought and received approval from Baylor's Recombinant DNA Subcommittee to develop a live virus vaccine lacking a portion of the viral genome coding for thymidine kinase, an enzyme that allows the pseudorabies virus to invade the nervous system of infected animals. But Baylor maintains that Kit never sought permission to test the vaccine in pigs either on or off the Baylor campus. Moreover, the subcommittee concluded that because recombinant DNA techniques were used in developing the vaccine, it must be regarded as a recombinant DNA virus, containing recombinant DNA molecules.

Responding to the subcommittee's findings, Kit argues that his virus does not violate NIH guidelines because it is not an organism "containing recombinant DNA". Kit acknowledges that recombinant DNA techniques were used to develop the vaccine, but since the alteration is a deletion rather than an insertion, the vaccine itself should not be considered a recombinant DNA virus. "If you look at a large enough number of thymidine kinase mutants, you'd find one with deletions", says Kit.

Kit and McConnell received permission to conduct the inoculations on the Maddox farm from the Texas Animal Health Commission. But Baylor president William Butler, in a statement accom-

panying the subcommittee report, noted that Baylor requires all faculty research to adhere strictly to NIH guidelines, regardless of the state or federal guidelines.

But Kit also rejects charges that the field test constituted an environmental release, since earlier experiments had shown that vaccinated animals were unlikely to shed the virus, transmitting it to other animals. Indeed, Kit maintains that no such transmission has occurred in any of the animals on the Maddox farm in the two years since the experimental vaccinations. Pseudorabies was eliminated on the Maddox farm following inoculations with Kit's vaccine.

For its part, NIH has established a nine-

member committee to investigate the 1984 incident. If the NIH committee finds a violation has occurred, it could recommend that all funds for recombinant DNA research be withdrawn from the two universities involved, although a lesser punishment seems more likely.

Kit's pseudorabies virus is currently being marketed by Biologics Corporation of Omaha, Nebraska, under the trade name Omnivac. Last month, in response to criticisms of its approval process, USDA requested that Biologics Corporation suspend sales of Omnivac while an environmental assessment was prepared. By the end of the month, USDA gave Biologics Corporation the green light to resume sales, but Jeremy Rifkin's Foundation on Economic Trends has brought suit to block the continued sale of the new vaccine.

Joseph Palca

Engineered plants

Another turn for ice-minus bugs

Washington

THE US Environment Protection Agency (EPA) was expected this week to make a decision about an Experimental Use Permit (EUP) application submitted by Stephen Lindow of the University of California (UC) at Berkeley to perform field tests of a genetically altered strain of *Pseudomonas syringae* designed to protect potato crops from frost damage. Approval seemed likely at the beginning of this week, but critics of genetic engineering were trying a series of last-minute manoeuvres to block the issue of the permit.

A Scientific Advisory Panel of independent researchers asked by EPA to evaluate Lindow's EUP had no quarrel with any of the proposed protocols or testing procedures. Panel member Robert Colwell, also of UC Berkeley, said the panel found Lindow's testing extremely thorough.

The strains of *P. syringae* developed by Lindow lack a portion of the genome coding for an ice-nucleating protein that acts as a focus for ice crystal formation, thereby causing frost damage to crops at warmer temperatures than if the protein were absent. The "ice-minus" bacteria that Lindow will be testing are conceptually similar to a strain of ice-minus *P. syringae* developed by Advanced Genetic Sciences (AGS) to protect strawberry plants. The AGS experiment is in abeyance while the company repeats pathogenicity tests.

Lindow has been seeking approval to test his bacteria since 1982. The National Institutes of Health (NIH) Recombinant DNA Advisory Committee (RAC) approved the field test in 1983, but a lawsuit brought by Jeremy Rifkin's Foundation on Economic Trends blocked the experiment. In 1984, with the lawsuit still pend-

ing, EPA assumed jurisdiction from NIH for approving such experiments.

A final settlement, reached just last month, absolves RAC of legal responsibility for approving Lindow's experiment, clearing the way for EPA to issue an EUP. Rifkin is still trying to block the Lindow experiment by asking EPA to issue a rule regarding liability insurance for those releasing genetically altered organisms into the environment. Rifkin claims UC's insurance is inadequate to cover the experiment.

The problems experienced by companies and researchers anxious to test genetically altered organisms has brought about a heightened awareness of the need for good public relations. Lindow plans to test his bacteria on a farm leased by UC in Tule Lake, California, a remote town near the Oregon border. As Tule Lake is subject to frost even in May, its agricultural interests should benefit if Lindow's field trials are successful.

Monsanto, also awaiting approval for an EUP for a genetically altered organism, has waged its own large public relations campaign. The company has sent thousands of brochures to residents of St Charles County in Missouri where the field test is to take place, and has held numerous meetings with area residents. Monsanto is hoping to test an altered *Pseudomonas fluorescens* containing a *Bacillus thuringiensis* endotoxin lethal to corn crop pests. An EPA decision on Monsanto's EUP was expected this week, but has been delayed for at least a week, according to EPA spokesman Al Heier.

Some light may be shed on the issues relating to experiments with genetically engineered organisms when the federal government publishes its expected policy statement on the subject.

Joseph Palca

Future of UK observatory

SIR—We are the group of astronomers of the Royal Greenwich Observatory (RGO) who are most directly responsible for the scientific care of the Anglo-Dutch telescopes on La Palma and we want to comment on the proposed move of RGO to the site of the Royal Observatory in Edinburgh (ROE), to the University of Cambridge or to the University of Manchester. Such a move would, at a crucial time, divert resources from the La Palma programme, from astronomy and from science, for benefits that are acknowledged to be intangible; we are opposed to a move of RGO.

However, given the assumption that the Science and Engineering Research Council (SERC) is now committed to move RGO, we believe that the council has an opportunity for a fresh approach to the organization of government astronomy in the United Kingdom. We hope SERC will build an institute which looks to the future of British optical astronomy. We hope that SERC will not, effectively, merge two government establishments in a way that perpetuates the existing way of working, the very mode which has been called into question. We see clearly that there are advantages to British astronomy of relocating RGO into an intellectual atmosphere, truly onto a university campus, and we prefer Cambridge for three reasons.

(1) We are very concerned about the effect of any move on the 4.2-m Herschel Telescope programme. We view with alarm the loss of productivity that has already been a baleful effect of the relocation issue, with literally hundreds of thousands of pounds' worth of time lost in anxious discussion before the move is even well-defined. The Herschel Telescope is the most ambitious project in UK optical astronomy, and it is now vulnerable because relocation has been made an issue at this unfortunate time. The further RGO is moved from its present home, the higher the proportion of key staff who will drop out of the Herschel Telescope project before it is completed. Even when the telescope is completed, we know from our experience with the other La Palma telescopes how essential it is to retain continuity of engineering staff in maintenance and development. A move within southern England to Cambridge would minimize disruption for La Palma.

(2) We have strong links with our colleagues in the British, Dutch, Irish and Spanish universities. We are determined to maintain these links if the move goes ahead to whatever university campus. We want to retain RGO's identity as an independent establishment with a duty to run La Palma for the whole of the astronomical community with which we are

proud to collaborate and which we are proud to serve. In order to do this effectively, we and our co-workers need to be able to attend meetings, to listen to what our colleagues have to say and to share our knowledge of La Palma with them. Our colleagues need access to the facilities with which we back up our overseas telescopes. It is important for RGO to be centrally placed in the United Kingdom, with easy access to and from Europe. Despite our present proximity to the major airports which serve Europe (and La Palma), and to motorways that connect us to our colleagues in Britain, some of our university colleagues think Sussex is inaccessible. It is inconceivable that a move to Edinburgh would improve RGO's accessibility for the broad astronomical community. Of the stated options, only Cambridge and Manchester are central enough to justify in this respect a move from Sussex.

(3) The reason that we exist as a group is to stimulate the building programme for La Palma instruments and the operation of La Palma telescopes from the point of view of astronomy. The move from Sussex would increase this stimulation only if the move were to an excellent university with the best and largest astronomy departments and the most active engineering and instrument science programmes. Cambridge offers the most direct access by the La Palma project to a broad spread of good relevant departments, including outstanding optical, radio and theoretical astronomy institutes. Cambridge is better for SERC's declared aim of improving the interaction between RGO and the universities because the astronomy, physics and engineering departments are closely grouped and near to the industrial science park.

If the move of RGO goes ahead, we strongly favour Cambridge over the other two possibilities that have been proposed, and we are strongly opposed to a move to Edinburgh.

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Freedom of thought

SIR—We refer to recent correspondence from C.K. Pallaghy (*Nature* 320, 9; 1986) of the School of Biological Sciences of this university stating his views on the size of Noah's Ark. Detailed refutations of such statements and conclusions, offered in

support of the creationist position of Dr Pallaghy and others, have been provided in many sources in recent years and we do not intend to canvass them yet again. We do not deny the right of a tenured academic to espouse whatever opinions he believes worthy of promulgation, through whatever extracurricular forum he is able to capture.

We do, however, dissociate ourselves absolutely from the creationist views expressed by Dr Pallaghy in *Nature* and elsewhere, and we wish it to be placed on record that Dr Pallaghy's creationist pronouncements are strictly his own personal opinions and are not part of our school's curricula. The other staff members of the School of Biological Sciences of La Trobe University remain totally committed to rational, scientific explanations of biological and all other natural phenomena.

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Theoretical choices

SIR—In his book review "Cortical convolutions" (*Nature* 320, 689; 1986), Colin Blakemore implies that I reject "pure empiricism" and hold that it would be desirable to have "prior agreement on a systematic and exhaustive exposition of all possible hypotheses". This is not quite right. I have, it is true, argued at length that "pure empiricism", in the form in which it is advocated by Popper, for example, is untenable. Scientific theories cannot be selected *solely* on the basis of empirical success and failure, just because this would not enable us to reject infinitely many grossly non-explanatory but empirically successful theories. In science, two criteria always govern choice of theory: (1) empirical success and failure, and (2) degree of "simplicity" or "explanatoriness". As the mature Einstein always emphasized, science cannot proceed without the assumption that the Universe is comprehensible, in some way or other, comprehensive or explanatory theories thus deserving more serious consideration than incomprehensible, non-explanatory theories (sometimes even in the teeth of empirical considerations).

None of this, however, implies that it is possible to give a "systematic and exhaustive exposition of all possible hypotheses". In my view, in general, this is not possible.

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Initial observations of fallout from the reactor accident at Chernobyl

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The fallout from the nuclear reactor accident at Chernobyl has been monitored at Studsvik, the Swedish energy research station located 75 km south of Stockholm. Here we present initial results of several different types of measurement. Gamma-ray spectrometry of samples of air, grass and milk reveals sixteen different nuclides, mainly volatile fission products. Radioactive particles up to $\sim 1 \mu\text{m}$ in diameter have been collected and analysed by electron microscopy; their form and composition lead to an estimate of $\sim 2,500^\circ\text{C}$ for the temperature in at least part of the reactor core. The level of radioactivity in air has decreased from initial values of a few becquerels (disintegrations per s) per m^3 , to very low values, and the activity level on grass is also decreasing due to decay and weathering. The radiation doses due to the fallout will be small compared with those from natural sources (such as radon emission in buildings), and will not cause significant health effects.

Early on 28 April 1986, an increased level of radiation was observed at the hot cell laboratory at Studsvik, located on the shore of the Baltic Sea, 75 km SSW of Stockholm. Rate meter readings increased from the normal 5–10 counts per second to 20–150 counts per s; higher than normal background values were also observed at every other place monitored at the site. During the morning hours a message was received that increased radiation had also been observed at the Forsmark nuclear power plant, 100 km north of Stockholm. Before noon we observed that cars and persons coming into the Studsvik site from places outside were contaminated. A source outside Sweden was judged to be the reason for the contamination. A $^{137}\text{Cs}/^{134}\text{Cs}$ ratio of 2 measured on air filters, combined with a knowledge of the wind direction, allowed us to confirm the suggestion that the contamination was caused by a release from a nuclear reactor east of Sweden and not from nuclear weapons.

On the ground there was a general twofold increase in rate meter count rates, and hot spots (particles) were found with a frequency of ~ 1 per m^2 . The dose rate increased from normal figures of $0.10\text{--}0.15 \mu\text{Sv h}^{-1}$ to $0.25\text{--}0.30 \mu\text{Sv h}^{-1}$ ($1 \text{ Sv} = 100 \text{ rem}$). Later we concluded from a continuously recording meter that the cloud had reached Studsvik on 27 April 1986 at 14:00 h local time.

Three stations just outside Studsvik and one inside are equipped with particulate-in-air filters (air flow rate $1.5 \text{ m}^3 \text{ min}^{-1}$). The sampling and analysis frequency was immediately increased to about three times per day, and samples of grass, milk and water were taken. Samples of hot spots were analysed by γ -ray spectrometry and electron microscopy; autoradiography was also tried and found valuable. Occasionally air was sampled through a particulate filter in combination with charcoal cartridges, to study the form of iodine present. Our colleagues (O. Karlberg and K. Gott, personal communication) also made *in situ* γ -ray measurements on the ground, in soil, field and grass areas, in order to study the effect of weathering (see below).

Sixteen different nuclides (^{137}Cs , ^{134}Cs , ^{131}I , ^{95}Zr , ^{95}Nb , ^{103}Ru , ^{106}Ru , ^{140}Ba , ^{140}La , ^{144}Ce , ^{133}I , ^{141}Ce , ^{239}Np , ^{132}Te , ^{132}I and ^{136}Cs) were identified in air by use of particle filters. The same nuclides also appeared in grass samples, but in somewhat different proportions (see Table 3).

The concentration in air was fairly stable during the first two days (28–29 April 1986). Typical values were $2\text{--}5 \text{ Bq m}^{-3}$ of ^{137}Cs activity and $6\text{--}12 \text{ Bq m}^{-3}$ of ^{131}I ($1 \text{ Bq} = 1$ disintegration per s). The figure for ^{131}I does not include gaseous iodine (see below). The sum of the activity of the 16 nuclides was $\sim 10 \text{ Bq m}^{-3}$. The maximum concentrations observed at a height of $\sim 400 \text{ m}$ (R. Fink, personal communication) were ~ 10 times higher than at ground level, in accordance with reports of fire and flames at the reactor.

Figure 1 shows an autoradiograph of a particle filter which was exposed to $\sim 200 \text{ m}^3$ of air. Many hot spots are present, of differing source strength. After the first three days, the concentrations in air dropped by two orders of magnitude.

On 2 May only small concentrations of ^{137}Cs , ^{134}Cs , ^{131}I and ^{132}Te were observed; for example, the concentration of ^{137}Cs was only 2 mBq m^{-3} . An increase was observed a few days later, and late on 8 May the levels had almost recovered to those initially recorded, but one day later they decreased again, by two orders of magnitude.

Particles which caused hot spots on autoradiographs (extra activity typically 20–150 counts per s, but occasionally up to 1,000 counts per s) showed a nuclide composition different from that of air filters and normal grass samples. One type of particle contained mainly ^{103}Ru ; another was composed almost entirely of ^{140}Ba and ^{140}La (Table 1).

Electron microscopy of hot spots revealed spherical particles, $\sim 1 \mu\text{m}$ in diameter (Fig. 2); some particles were smaller than this. The regular form may indicate that melting has occurred. The diameter of a solid particle with the composition of particle 1 (Table 1) would be $\sim 1 \mu\text{m}$: we have therefore found 'pure' ^{103}Ru – ^{106}Ru particles. Ru has a melting temperature of $2,500^\circ\text{C}$, indicating that part of the reactor core reached this temperature.

From the observation that the ratio of ^{137}Cs to ^{134}Cs was 2.0 in the air filters, we conclude that, on average, the fuel had been in

Table 1 Selected hot-spot particle compositions

Nuclide	Activity (Bq)		
	Particle 1	2	3
^{103}Ru	10,000	6,900	
^{51}Cr	1,700		
^{99}Mo , ^{99m}Tc	1,600		
^{106}Ru	2,800	1,800	40
^{132}Te	810	20	
^{131}I	70	60	25
^{140}Ba – ^{140}La			2,400
^{95}Zr – ^{95}Nb			10
^{141}Ce			75
^{144}Ce			120
^{239}Np			70



Fig. 1 Autoradiograph of particle filter exposed to $\sim 200 \text{ m}^3$ of air on 28 April 1986.

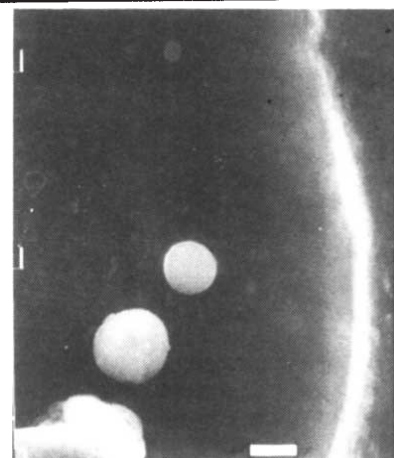


Fig. 2 Electron micrograph of spherical particles from an autoradiograph 'hot spot' (extra activity 200 counts per s). Scale bar, $2 \mu\text{m}$.

Table 2 Inventory of a 3,000-MWth reactor core operated for 400 days and nuclide ratios normalized to ^{137}Cs

Nuclide	Inventory (10^{17} Bq)	Ratios in core	Ratios in air filters*	Ratios in grass†
^{137}Cs	0.52	1	1	1
^{131}I	10	19	5 (20)	35–40
^{103}Ru	14	26	0.17	0.8–2
^{141}Ce	18	34	0.08	1.5
^{132}Te	14	26	0.7 (1.5)	4–5
^{239}Np	186	360	0.5 (1.0)	6–10

*From one typical filter; values in brackets are corrected for gaseous iodine or decay. †In a few initial samples.

Table 3 Fission product activities in grass and air

Nuclide	Munich grass, 6 May (kBq m^{-2})	Crete grass, 7 May (kBq m^{-2})	Studsvik air*, 30 Apr–1 May (Bq per sample)
^{131}I	4.0	2.8	0.14
^{137}Cs	1.4	0.11	0.11

*An air sample from a particle filter is included for comparison of nuclide ratios.

operation for ~400 days. The nuclide inventory for a 3,000-MWth (thermal megawatt) reactor after 400 days of operation is given in Table 2, along with the ratios in the core, in air filters and in grass, normalized to ^{137}Cs . From this we can confirm that, as expected, the volatile nuclides (^{131}I , ^{137}Cs , ^{132}Te) are more dominant in the fallout than non-volatile elements (^{103}Ru , ^{141}Ce , ^{239}Np); however, the proportion of non-volatile nuclides is surprisingly high, indicating very high temperatures in the reactor core and the absence of retaining barriers.

Samples of particle air were also taken by means of a particle filter followed by an impregnated charcoal bed which collects gaseous iodine. This technique revealed that, in samples taken from 30 April to 2 May, 75–80% of the iodine was gaseous or desorbable from particles. In samples from around 6 May, the gaseous and desorbable iodine was still dominant.

Assuming steady reactor operation for a time considerably exceeding 8 days, the ratio $^{133}\text{I}/^{131}\text{I}$ in the fuel will have had its steady-state value of 2.14. This ratio decreases after a shut-down, due to the more rapid decay of ^{133}I than of ^{131}I . Backward extrapolation indicates that the chain reactions in the core stopped at ~19:00 on 25 April (Studsvik Lr) (see Fig. 3).

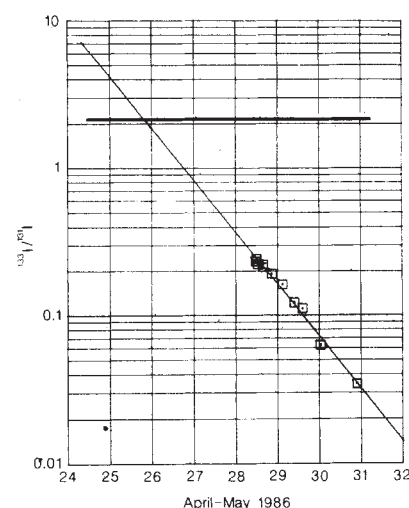
The normal background dose rate at Studsvik is $0.10\text{--}0.15 \mu\text{Sv h}^{-1}$. During 28 April these values increased to $0.20\text{--}0.30$; that is, to about double the background value. The dose rate remained fairly stable for a few days; then, a slight decrease was observed to ~0.20 on 2 May. In small areas north of Stockholm which had some rainfall, the dose rate increased to 50–100 times the background; that is, to $5\text{--}10 \mu\text{Sv h}^{-1}$.

Grass samples were taken at many places after we noticed the high concentrations of fission products in air. In the Studsvik area, which has not experienced any significant rain, the dry deposition on grass was $20\text{--}80 \text{ kBq m}^{-2}$ of ^{131}I and $0.5\text{--}2 \text{ kBq m}^{-2}$ of ^{137}Cs during the final days of April. Typical figures for the region around Studsvik were $5\text{--}20 \text{ kBq m}^{-2}$ of ^{131}I in the first week of May. For the southern part of Sweden, lower values have been reported and at some other places north of Stockholm, higher values are recorded (see Table 3).

Typical values of ^{131}I in milk around Studsvik were 10 Bq l^{-1} , due to the concentration in the cows' breathing air (they were not grazing). The Swedish Radiation Protection Institute has recommended that farmers let cattle stay indoors until the deposition on grass is less than $\sim 3 \text{ kBq m}^{-2}$, corresponding to 2 kBq l^{-1} of milk. The corresponding German limit is 500 Bq l^{-1} .

Potential doses from the food chain grass–cow–milk have been calculated for the nuclides ^{131}I and ^{137}Cs . The basis for the calculations is the measured deposition of the nuclides on the grass. For the initial dose, we use the equation $D_{\text{milk}} = CAF_m UD_t$, where C is the deposition on grass (in Bq m^{-2}), A is the daily

Fig. 3 $^{133}\text{I}/^{131}\text{I}$ ratio in particle filters at Studsvik plotted against time. Extrapolation of the data back to $^{133}\text{I}/^{131}\text{I} = 2.14$ (bold horizontal line) gives the time of reactor shut-down (see text).



grazed area ($= 100 \text{ m}^2$), F_m is the distribution factor, which is the fraction of daily intake which at equilibrium is likely to be recovered per litre of milk (day l^{-1}), U is the daily milk consumption (l day^{-1}) and D_i is the weighted committed dose equivalent (Sv Bq^{-1}). For ^{131}I , we take $C = 1,000$, $F_m = 10^{-2}$ (ref. 1) and $D_i = 1.1 \times 10^{-7}$ (ref. 1); for ^{137}Cs , $C = 100$, $F_m = 7 \times 10^{-3}$ (ref. 2) and $D_i = 1.4 \times 10^{-8}$ (ref. 3).

For ^{131}I the value of D_i is that for children who, because of their anatomical and metabolic characteristics, comprise the most vulnerable part of the population. The doses to children from ^{137}Cs will be higher than for adults. If for safety the dose conversion factor to children is assumed to be a factor of 10 higher than for adults, the dose to children will be a factor of ten higher than the value given below.

The initial concentration on vegetation will decrease due to both radioactive decay and natural removal. The latter is described by a half-life with a typical value of 14 days (ref. 1). This leads to an effective decay constant, λ_e , of 0.13 day^{-1} for ^{131}I and $\sim 0.05 \text{ day}^{-1}$ for ^{137}Cs . The dose commitment for the assumed contamination of grass will thus be 0.8 mSv for ^{131}I and 0.02 mSv for ^{137}Cs . The assumed daily milk consumption is 1 litre per day. Rainfall may give rise to more rapid weathering, which is what we have observed. ^{137}Cs on the ground can also cause external exposure. The dose conversion factor is $2.5 \times 10^{-12} \text{ Sv h}^{-1} \text{ per Bq m}^{-2}$, which will give a dose rate of $6 \times 10^{-8} \text{ Sv day}^{-1}$ for a contamination of 1 kBq m^{-2} of ^{137}Cs .

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Early estimates of UK radiation doses from the Chernobyl reactor

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The plume of radioactive material from the Chernobyl reactor accident passed over the United Kingdom and will increase the radiation dose to the population in the coming year. The increase above the normal annual dose from natural radiation, averaged over persons of all ages, will be about 15% in the north and 1% in the south of the country. Averaged over all ages and areas, the increase will be about 4%. This excess dose will decrease substantially in subsequent years.

The accident at the nuclear power station in Chernobyl, near Kiev, on or after 26 April 1986, led to substantial quantities of

radioactive material being released to the atmosphere. Wind initially transported the material towards northern and western Europe. Activity was first detected in the southern United Kingdom, some ~2,000 km away, on 2 May.

The National Radiological Protection Board (NRPB), the operator of nuclear installations and the regulating authority, had anticipated this eventuality and had intensified its normal programmes of environmental monitoring. During the following days many measurements were made and a considerable amount of data were generated throughout the country. NRPB was assigned responsibility for collating and evaluating these results: the initial information is used here to make a preliminary estimate of the radiation doses to the population of the United Kingdom.

At Chilton, the concentration of airborne particulate activity increased to a maximum during the afternoon of 2 May, declined overnight to about one-tenth of the maximum value, and by a further factor of ten during the following day. The major radionuclides detected were ^{131}I , ^{132}Te , ^{132}I , ^{103}Ru , ^{106}Ru , ^{134}Cs , ^{137}Cs , and ^{140}Ba and ^{140}La . Table 1 lists the integrated concentrations of these radionuclides for the 24 hours during the passage of the cloud over Chilton; these values are typical for the United Kingdom as a whole. Early reports from various organizations indicated that the total concentration of radioiodine was somewhat greater than that indicated by the particulate material collected on filters, and this was borne out by subsequent environmental measurements. In the northern United Kingdom, the peak concentration in air was attained later than in the south, and lower levels persisted somewhat longer.

Deposition of radioactive material across the country was very variable, depending mainly on whether it rained. Generally, there was less rain in the southern United Kingdom during the passage of the cloud, whereas the north-west of England, North Wales, Scotland and Northern Ireland had sporadic heavy rainfall. In the following assessment we distinguish between the two parts of the country, calling them for convenience 'south' and 'north'. Concentrations of activity in rainfall were quite variable and a temporary ban on the consumption of freshly collected rain water was recommended in some areas by NRPB and the Department of the Environment.

The radioactive material deposited in the south was mainly ^{131}I , resulting from dry deposition. In areas where rainfall occurred during the passage of the cloud, not only were several other radionuclides detected on grass, but the levels were much higher than in the south.

The radionuclides of greatest importance are ^{131}I , ^{134}Cs and ^{137}Cs , as these are transferred through food chains to humans. Milk is an important early indicator of such transfer. At Chilton, milk collected from a nearby farm was found to have a small but

Table 3 Representative doses in the year May 1986 to April 1987 (μSv)

Mechanism of exposure	South		North	
	1-yr-old	Adult	1-yr-old	Adult
External irradiation by the passing cloud	0.1	0.1	0.1	0.1
Inhalation	7	8	7	8
β -Contamination of the skin	0.03	0.03	1	1
γ Rays from deposited activity	10	10	160	160
Ingestion	30	4	700	100
Dose from all mechanisms (rounded)	50	20	900	300
Dose weighted for age distribution	20		300	
Dose weighted for population distribution			70	

Doses refer to actual effective dose equivalents or committed effective dose equivalents for the year after the accident.

measurable quantity of ^{131}I as early as 2 May, confirming that ^{131}I in vapour form had preceded the cloud of particulate material; the concentration reached a peak of 54 Bq l^{-1} ($1 \text{ Bq}=1$ disintegration per s) on 6 May and has subsequently declined with an effective half-life of about 2 days. Because of the lower deposition of caesium isotopes at Chilton, these were not so easily measurable: ^{137}Cs reached a peak of $\sim 2 \text{ Bq l}^{-1}$ and is only now, a week later, declining. Elsewhere, where rainfall had been the dominant mechanism of deposition, the representative peak concentration of ^{131}I in milk was $\sim 400 \text{ Bq l}^{-1}$; the concentrations of caesium isotopes reached peak values of $\sim 400 \text{ Bq l}^{-1}$ for ^{137}Cs and 200 Bq l^{-1} for ^{134}Cs (see Table 2).

Leafy vegetables are also a major contributor to the dietary intake of iodine and caesium isotopes. We made some measurements on vegetables obtained locally, and more information was received from other areas of the country (see Table 2).

When taken into the human body, iodine concentrates in the thyroid gland, and measurements of ^{131}I in this organ provide the most reliable means of estimating doses. Our early measurements indicate that the thyroid content of ^{131}I resulting from the accident is measurable in some persons in the southern part of the United Kingdom, but we still have insufficient data to use as a basis for dose calculations. Our current estimates are therefore based on environmental measurements.

There are several mechanisms of human exposure to the contaminants from Chernobyl. In the sequence in which they deliver dose, they are as follows: external irradiation by the passing cloud; inhalation of radioactive material in the cloud; β -ray contamination of the skin; external irradiation by material deposited on the ground; ingestion of contaminated foods.

To determine the external radiation dose from the passing cloud, we assumed that the representative peak value persisted for 24 hours over the whole country. This enabled us to use standard conversion factors from activity concentration to effective dose equivalent¹, in which allowance is made for the different effects of photons and electrons, and a weighting factor of 0.01 is adopted for risk to the skin². We also made an allowance for the likely contribution of the noble gases based on an estimate of relative release³. We deem the doses to be the same for children and adults.

For doses from inhalation, we have used the integrated concentrations in air from Table 1 for the particulate β - and γ -emitting radionuclides. We estimated the concentration of non-particulate ^{131}I from preliminary information and used concentrations of actinides in air from a single observation. We used standard values of inhalation rate⁴ and dose per unit intake⁵ for children and adults to determine the committed effective dose equivalents in Table 3.

There are no field data for the deposition of activity on human skin, so we had to take a cautious approach. Our assumption was that the deposition on grass reflects the deposition on people out of doors and that the contamination lasts for 12 hours. We then

Table 1 Radionuclides in United Kingdom environmental samples

Radionuclide	Integrated concentration in air at Chilton (Bq s m^{-3})	Representative values for initial deposition on grass (Bq m^{-2})	
		South	North
^{131}I	1.6×10^5	500	8×10^3
^{132}Te – ^{132}I	1.9×10^5	200	6×10^3
^{103}Ru	1.2×10^5	50	3×10^3
^{106}Ru	4.5×10^4	—	—
^{134}Cs	3.2×10^4	20	3×10^3
^{137}Cs	7.6×10^4	30	5×10^3
^{140}Ba – ^{140}La	2.4×10^4	10	2×10^3

Table 2 Representative activity concentrations in milk (Bq l^{-1}) and leafy vegetables (Bq kg^{-1})

Radionuclide	South		North	
	Milk	Vegetables	Milk	Vegetables
^{131}I	50	100	400	200
^{134}Cs	4	10	400	100
^{137}Cs	2	5	200	50

Making radiation understood

Governments and nuclear energy agencies are resolving to put more energy into explanation. But they have an uphill task ahead of them.

If the past two weeks have shown anything at all, it is that the world remains in need of a general awareness of radiation and of its potential consequences. Nothing else can account for the near-panic that seems to have afflicted some of those living in Western Europe since the news from the Ukraine came to light.

One of the beneficial consequences of the Chernobyl accident is that many of the organizations concerned have now taken to saying that they will do more to ensure that non-technical people are more accurately informed. Past experience will remind them that it is a daunting task.

One incidental problem is that first-hand knowledge of radiological protection of the sophistication required intelligently to follow events such as those of the past few weeks is rare. Most of the practitioners are people who began their professional lives as nuclear physicists, radiochemists or radiographers, although the needs of the nuclear industry explain why there are now higher education courses, usually at the graduate level.

A further complication is that of the units in which radiation is measured. In the lifetimes of many of those with a second-hand knowledge of the field, the becquerel (representing one disintegration

of any kind per second) has, for example, replaced the curie as the unit of activity ($1 \text{ Ci} = 3.7 \cdot 10^{10} \text{ Bq}$). As the excitement of the past two weeks has shown, the Bq is dramatically too small a unit, small enough to yield large numbers in quite unremarkable circumstances; the natural content of radon-222 in the air may for example range upwards from an estimated 1.8 Bq m^{-3} at equilibrium to 30 times as much in granitic areas.

Confusingly (for those whose knowledge is second-hand) the other units newly introduced in 1978 are too large. The modern equivalent of the rad, itself a measure of radiological energy absorbed per unit of matter, is too large (1 gray, or Gy, = 100 rad = 1 joule of energy per kilogram). The same goes for the Sievert, the modern equivalent of the rem, the modification of the unit called the rad that allows for the different effectiveness of different kinds of radiation in living tissues. People used to manipulating numbers are able to comprehend and use these relationships; most people are not, and there are no simple yardsticks.

The complexity of the radiation problem is another hurdle to be crossed. Environmental radiation can cause trouble either because it exposes living

things to what lawyers call assaults from outside, or because it is ingested in a general sort of way or because particular radionuclides are absorbed into different tissues (as iodine is into the thyroid or plutonium into bone). It will unfortunately be a long time before these distinctions are widely appreciated.

Much worse is true of the attempts that professional people make to allow for the stochastic nature of most of what they say about the effects of radiation. Why, for example, work out the "dose commitment" of a population as a consequence of iodine-131 from Chernobyl in units called man-Sieverts? Because somebody (rightly) along the line has made the assumption that the biological consequences are linear functions of the dose. But since the biological consequence is most often a cancer, in simple language something that you have or do not have, the generally unfathomable concept of probability is unavoidable. It is a little like trying to persuade a man on an omnibus that he is safe because the chance of the vehicle being blown over in the wind is merely one in a few tens of millions (which is precisely what the authorities will now have to attempt with nuclear power stations). It will be an uphill task.

computed the β -ray dose to the skin for people in the south and north by standard techniques and transformed this to effective dose equivalent⁶. If we were to allow for the likelihood of a person being indoors during the passage of the cloud, the estimate in Table 3 would be reduced by an order of magnitude.

A considerable number of γ -ray dose-rate measurements were made throughout the country. The results were analysed in terms of net dose rate 1 m above the ground. For the southern areas, the representative peak value was $0.1 \mu\text{Sv h}^{-1}$; for the north, $0.6 \mu\text{Sv h}^{-1}$ ($1 \text{ Sv} (= 1 \text{ J kg}^{-1}) = 100 \text{ rem}$). These dose rates were then apportioned according to the relative contributions of the deposited radionuclides⁷, and the dose over the year was estimated, the assumption being that radioactive decay is the only influencing factor. We then applied an indoor occupancy factor of 0.9⁸, a body self-shielding factor of 0.7⁹ and a building shield factor of 0.1¹⁰ (see Table 3). It is worth noting that somewhat elevated dose rates will persist after next year. By May 1991, for example, the extra dose in the north will have been about $400 \mu\text{Sv}$ and in the south $20 \mu\text{Sv}$; the dose from natural background radiation in the same period will, on the average, be $7,500 \mu\text{Sv}$.

For ingestion, it is particularly necessary to differentiate between infants and adults. For the consumption of foods, we have used concentrations of ^{131}I , ^{134}Cs and ^{137}Cs that are broadly typical of the peaks found in our two geographical regions. We used consumption rates that are typical for the population as a whole⁴, and we have estimated doses from the representative peak concentrations⁶. The dose estimates are given in Table 3; they refer to the committed effective dose equivalent from the consumption of foodstuffs throughout the year after the accident. The standard dosimetric model for caesium isotopes⁵ is

based on values of metabolic parameters for adults; it will therefore overestimate the dose to young children. The contribution of the slight traces of activity in ordinary drinking water (ground or surface) is negligible and we assume that the ban on drinking fresh rain water was effective. Activity that will eventually find its way into animal carcasses and other foodstuffs will make only second-order contributions.

The dose estimates in Table 3 are for two age groups and our two geographical areas. Doses weighted for age and geographical distribution of the population are also given. For a United Kingdom population of 56 million, the weighted individual dose of $70 \mu\text{Sv}$ implies a collective dose of $4 \times 10^3 \text{ man Sv}$ in the year after the accident. This value is a few per cent of the annual collective dose to the population from natural radiation, which is about 10^5 man Sv every year.

We are grateful for the information provided to us by: British Nuclear Fuels PLC; the Atomic Energy Authority Establishments at Harwell, Winfrith and Dounreay; the Central Electricity Generating Board; the South of Scotland Electricity Board; the Ministry of Agriculture, Fisheries and Foods; the Department of the Environment; the Scottish, Welsh and Northern Ireland Offices; and our colleagues at Glasgow, Leeds and Chilton.

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Oceanography

Injecting ice-shelf water and air into the deep Antarctic oceans

from Stan Jacobs

ON page 233 of this issue¹ Peter Schlosser attributes the supersaturation of helium isotopes (^3He) in sea water at depths near 500 m on the Weddell Sea continental shelf to melt water derived from the base of the Filchner Ice Shelf. Because noble gases have low solubility in sea water, a deep maximum is induced in a profile of ^3He against depth by dissolved air that had been trapped in the ice during its formation. The ^3He -spiked melt water can be found subsequently in the bottom water that is generated in the Weddell Sea. Gas concentrations in the deep ocean may thus be directly influenced by air that has had a long residence time in the Antarctic ice sheet. Apart from introducing helium as a useful Antarctic tracer, Schlosser's work supports previous interpretations of 'ice-shelf water' — a water mass of potential interest to ocean ventilation and to the mass balance of the Antarctic ice sheet.

It has long been appreciated that the large Antarctic ice shelves cool a portion of the Antarctic continental shelf waters to temperatures below the freezing point of the sea surface². This results from a decrease in the *in situ* freezing point of sea water with increasing pressure (depth), 0.07°C per 100 db (m), and from melting and vertical heat flux at the vast basal areas of the very cold floating shelf ice. Seawater sinking at a temperature near -1.9°C during ice formation in austral winter is thus up to 0.5°C warmer than the melting temperature of ice at 200–700 m depths. The vigour of the sub-ice shelf mixing and circulation will determine how effectively this excess heat can be delivered to the ice/water interface. Temperature remains the simplest tracer of shelf-ice influence, but does not reveal whether the coldness of ice-shelf water results from heat conduction into the ice, perhaps accompanying basal freezing³, or from melting ice of continental or marine origin.

More recently, oxygen isotope (^{18}O) measurements in sea water have revealed the effects of glacial melt water near Antarctica^{4,5}. The extreme depletion of ^{18}O in glacial ice against sea water makes it a potentially excellent tracer of melt water from the continental ice. One drawback of ^{18}O as an oceanographic tracer in the Antarctic is that sea-surface precipitation (including snow blown off the continent) can be almost as depleted in ^{18}O as is the shelf ice. If this precipitation is preferentially funnelled to continental shelf polynyas (open areas in the winter pack ice where intense air/sea interactions

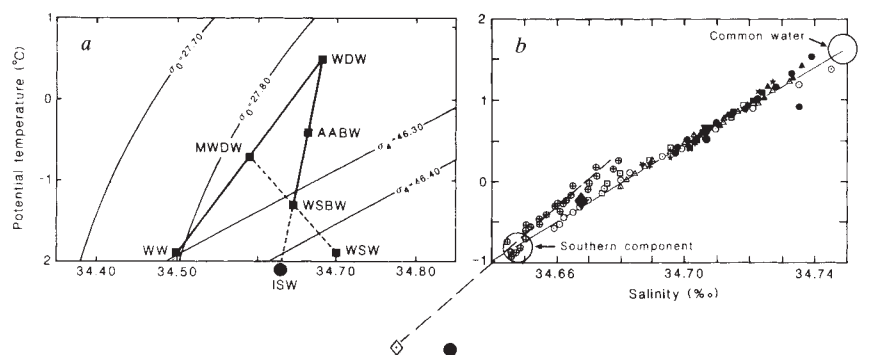
occur), it may be incorporated into the sinking, high-density shelf waters, producing a local resemblance to glacial melt water. What preserves the stronger ^{18}O , temperature and ^3He signals in ice-shelf water is the apparently stable stratification of most of the water column on the continental shelf. Rapid horizontal mixing of melt water into the stabilizing halocline (salinity gradient) gives ice-shelf water its identity, which is retained during northward spreading along isopycnal (constant density) surfaces on the shelf.

Ice-shelf water has largely been ignored as a component in the formation of bottom water: its volume was calculated to be small; it appeared to recirculate on the continental shelf; and bottom-water characteristics could be accounted for by various combinations of other water masses. Ice-shelf water does reach the

needed to provide the near-surface characteristics.

Antarctic bottom waters form year-round near the continental-shelf break from deep and shelf waters, some of which have detoured beneath the ice shelves. Because of this subsurface mode of generation, which involves components with little or no equilibration time at the sea surface, the effective ventilation rate may be lower for deep water now formed in the Antarctic than in the North Atlantic. During the last glacial period, the Antarctic continental shelves were apparently filled with glacial ice and were not available as temporary storage areas for high-density shelf waters. But ice-shelf water might have continued to spike the bottom water during that time if the shelf ice was not fully grounded. Additional heat sources for ice-shelf melting exist today and presumably existed during glacial periods. They are the circumpolar deep water and its derivatives which intrude, sometimes in jet-like form, beneath the ice shelves^{3,6}.

From these analogies, it may be of interest to consider whether significant subsurface to deep ocean meltwater spikes were produced during glacial-to-



a, Typical temperature/salinity diagram (from Fig. 12 in ref. 7) used to illustrate how bottom water (Antarctic Bottom Water, AABW and Weddell Sea Bottom Water, WSBW) might be derived from other water types in the Weddell Sea. The solid circle added at -2.08°C and 34.628 parts per thousand (‰) represents average ice-shelf water (ISW) from Schlosser's Table 1 (ref. 1). It is apparent that the simplest bottom-water recipe would be a mixture of deep water (WDW) and ISW (but see ref. 5). **b**, A similar plot (from Fig. 4 in ref. 8) showing temperature/salinity characteristics for waters colder than 1.6°C in the circumpolar Antarctic oceans. Black symbols, Pacific sector; open symbols, Indian sector; crossed symbols, Atlantic sector. The Southern Component in **b** approximates the characteristics of WSBW in ref. 4, about midway between AABW and WSBW in **a**. The Atlantic Sector trend can be related to ISW, in particular to Schlosser's¹ three coldest values (open diamond).

continental-shelf break, and recent evidence shows that this water also spills off the shelf and flows along and down the continental slope⁶. Ice-shelf water might therefore be considered as the missing ingredient on Antarctic temperature/salinity diagrams (see figure). However, ice-shelf water may be more of a tracer than a major factor in bottom-water formation, unless its volume has been greatly underestimated or is compensated for by a high flux. In addition, other water types, in particular low-salinity shelf waters⁵, are

interglacial transitions where continental ice disintegrated in the ocean.

Much as oceanographers disregarded ice-shelf water, glaciologists have until recently paid little heed to the ocean, except perhaps as a medium in which icebergs could be floated away from the continent. Extending the work of Robin⁷, some estimates now show that as much as one third of the wastage side of the ice-sheet mass balance may be caused by basal melting of the ice shelves⁵. The existence of good meltwater tracers offers the

promise of their eventual use to refine presently crude estimates of melting. Chemical tracers are particularly valuable in this regard because they tend to integrate over various spatial scales, and in some cases can provide information on processes and timescales. For example, unpublished data on chlorofluoromethane (freon) from the Ross Sea suggest short residence times for sea water on the continental shelf and under the Ross Ice Shelf.

Schlosser's initial ^4He results need to be pursued by additional sampling and analyses. Fractionation effects when sea ice freezes and the different diffusivities of helium in ice and water may complicate the picture^{10,11}. Ice-shelf water is derived from shelf water that has obtained its characteristics during ice formation at the sea surface. Because helium is enriched in sea ice and depleted in sea water from which that ice forms, the shelf water would initially be depleted in ^4He . That

could change the size of the ^4He anomaly produced by the air in melting glacial ice, as could the melt water from basal sea ice. Perhaps the ^4He increase at the sea surface in Schlosser's Fig. 2a (ref. 1) illustrates the impact of melting pack ice on his austral summer profile. □

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The blood-brain barrier

New aspects to the function of the cerebral endothelium

from F. Joó

THE walls of the cerebral microvessels represent a cellular membrane between blood and brain with special physiological, biochemical and morphological properties¹. Metabolic coupling of the main functions of cerebral microvessels to the needs of the nerve and glial cells has been described² and certain characteristics of the molecular processes underlying the regulation of vascular tone and permeability have been noted³. Recent results, however, reveal new features of the cerebral vascular endothelium and help to build a comprehensive picture of the brain microvasculature and the ways in which it differs from that in the periphery.

Three main lines of research have revealed these important new features. First, it has been shown that cerebral endothelial cells synthesize and/or store neurotransmitter substances with vasoactive potency. Although certain neurotransmitters, such as noradrenaline⁴, serotonin⁵ or histamine^{6,7}, or enzymes involved in the synthesis of adrenaline⁸, γ -aminobutyric acid⁹ and acetylcholine¹⁰ have been detected biochemically in preparations enriched in brain microvessels, their presence was often thought to result from contamination with detached nerve endings. But more recently, Parnavelas *et al.*¹¹ have shown at the ultrastructural level that choline acetyltransferase, the enzyme that synthesizes acetylcholine, is confined to the vascular endothelial cells themselves and not to nerve endings innervat-

ing the vessel wall.

Since the effectiveness of histamine¹², serotonin¹³ and bradykinin¹⁴ in opening the blood-brain barrier has been demonstrated, it is reasonable to assume that the neurotransmitters which have vasoactive effects when released from the endothelial store during pathophysiological conditions like hypoxia can interact with specific receptors on the luminal or abluminal membranes, or both.

These receptors may be linked to the adenylate cyclase system¹⁵ or may be able to interact directly with cellular functions of the cerebral endothelium, such as regulation of pinocytosis or of the tightness of the junctions between endothelial cells. Interestingly, there seems to be no synthesis or accumulation of vasoactive peptides in the cerebral endothelium, despite the utmost importance of these peptides in the regulation of cerebral blood flow¹⁶ and the close structural relation of peptidergic nerves to the microvessel wall^{17,18}.

Second, the mode of migration of polymorphonuclear leukocytes across the blood-brain barrier shows that, in contrast to the peripheral vessels where during inflammation leukocytes migrate through the intercellular junctions, in the brain leukocytes leave via the transcellular route¹⁹.

It is also noteworthy that the cerebral vessels, apart from well-known differences in structural composition, differ from peripheral vessels in their response

to histamine. Peripheral endothelial cells contract in response to histamine whereas cerebral vessels show only an increase in pinocytotic activity¹². The significance of opening of tight junctions, which connect the neighbouring endothelial cells, has also been questioned²⁰. It appears that during osmotic opening of the blood-brain barrier, passage of macromolecules across the endothelium of brain capillaries is not by an interendothelial route but rather by a transendothelial one resulting from amplified vesicular activity.

The importance of endocytosis and transcytosis in the macromolecular transport of cerebral endothelium raises interest in the metabolic regulation of this process. It seems the intracellular generation of cyclic nucleotides in the endothelium is important. Consequently, drugs which interfere either with the generation or breakdown of cyclic nucleotides (or both) in the cerebral endothelium may, at the same time, increase pinocytosis and macromolecular transport. It is still unclear how the amplified vesicular activity leads in pathological circumstances to the formation of transendothelial channels, establishing the structural basis of macromolecular transport across the endothelial cells.

Finally, the interesting finding of Traugott and co-workers²¹ clearly suggests that the cerebral endothelial cells are involved in an active manner in the initiation and perpetuation of inflammatory demyelinating lesions in the central nervous system. With the use of a monoclonal antibody, these authors detected the expression of class II molecules of the major histocompatibility complex on the endothelial cells (and on the astrocytes) in multiple sclerosis lesions of different ages. Such

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expression is important for the presentation of antigen in the induction of a cellular immune response. Because of the presence of a blood-brain barrier, circulating macrophages are unlikely to be involved in the initial presentation of antigen within the central nervous system.

Because the expression of class II molecules can be induced on the endothelial cells by contact with activated T lymphocytes, it is probable that this results in the release of lymphokines that break down the blood-brain barrier and hence leads to the invasion of blood-borne cells into the brain parenchyma.

As sophisticated techniques are now available both for isolating microvessels from the brain tissue and for culturing cerebral endothelial cells²², it seems likely that new therapeutic means will soon be developed to influence cerebral endothelial functions. Examples of such methods are to improve the transendothelial transport of particular substances and to reduce albumin extravasation in vasogenic brain oedema. □

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Growth factors

From the laboratory to the clinic

from Mike Dexter

HORMONE therapy is used for a wide range of disorders and the results of treatment are often dramatic. The work primarily involves the classical 'glandular' hormones that are released as circulating messenger systems because fairly large amounts of material are readily available and their target cells and tissues are known. But many other important messenger systems modulate the behaviour and function of cells. Cytokines, for example, are soluble cell products that can modify the growth or behaviour of the same or unrelated cells. Thus the biological activities of lymphokines and monokines produced by lymphocytes and monocytes, respectively, have been elegantly elucidated *in vitro*. The problem is that these soluble mediators are often only produced locally and in vanishingly small amounts, making studies of their role *in vitro* and of their clinical usefulness formidable. But with the molecular cloning and expression in suitable vector systems of many of these molecules, sufficiently large amounts of material are now available to perform preclinical and clinical studies and this potential was highlighted at a recent meeting*.

It is all very well being able to manipulate cells *in vitro*, but clinical application hinges on whether or not these agents can exert effects *in vivo* and if the effects observed can be predicted from the *in vitro* analyses. Under discussion were growth factors regulating B and T lymphocytes and tumour necrosis factor. Several general points should be noted. First, recombinant molecules essentially have the same biological activities and are just as effective as the native molecules — irrespective of where, how or by whom they were cloned. Second, these cytokines do elicit responses *in vivo* — although sometimes with surprising consequences.

Third, many of the cytokines under consideration will almost certainly prove useful in the clinic, either used alone or in combinations — the problem is to define the disease, the dose and the route of administration.

Murine growth factors, interleukin-3 and granulocyte-macrophage colony stimulating factor (GM-CSF), which are active on stem cells or myeloid progenitor cells *in vitro*, will elicit responses *in vivo* (D. Metcalf, Melbourne; my own work; H. Broxmeyer, Indianapolis). Interleukin-3 activates stem cell cycling and promotes an increase in stem cells and progenitor cells in the spleen whereas GM-CSF can mobilize and recruit activated granulocytes and macrophages. Having established their activity *in vivo*, the next stage is to determine whether these agents, alone or in combination with macrophage colony stimulating factors (M-CSF) (P. Ralph, San Francisco), have therapeutic potential in enhancing blood cell generation in, for example, pre- or post-chemotherapy radiation, in the myeloid hypoplasias or in neutrophil deficiency syndromes. Furthermore, as macrophages and granulocytes play a major part in combating infestations with helminths, protozoa and numerous other colonizing organisms, the ability to activate the mobilize these cells with GM-CSF and M-CSF has many profound implications. Many such studies are already underway using animals as models and it must be only a matter of time before the first clinical trials are performed. In this respect, recombinant human erythropoietin, a factor that enhances red blood cell production and works *in vivo*, seems to be a suitable starting material (J. Egrie, Thousand Oaks, United States) — especially for those patients with anaemia caused by kidney damage.

A recurring problem with many of these cytokines is their rapid clearance from the

plasma coupled with the fact that cells must often be continuously exposed to the agents to elicit a response. But the pharmacokinetics are being studied and continuous infusion regimes are available to maintain a constant plasma level. This could be particularly important in the management of malignant disease. Interferon is very efficient for the treatment of hairy cell leukaemia (J. Quesada, Houston) and another cytokine (granulocyte-colony stimulating factor, or pluri-poiectin) has the remarkable property of being able to induce differentiation of myeloid leukaemic cells while at the same time amplifying the number of primitive normal progenitor cells *in vitro* (M. Moore, New York; E. Platzer, Erlangen). The potential use is obvious and the way is clear to undertake studies on the use of this regulator for 'leukaemia ablative therapy', *in vitro*, before autologous marrow transplantation (see Chang, J. *et al. Lancet* i, 294; 1986).

What about the interferons and other lymphokines? Clinical trials are underway to investigate the therapeutic potential of interferons in various malignant and non-malignant diseases. With the exception of hairy cell leukaemia, the results are somewhat disappointing (H. Oettgen, New York; P. Voldering, San Francisco; Robinson, Denver). But the lymphokines represent an interacting network of messenger signals capable of modulating the responses of one to the other. Interleukin-1, for example, stimulates myeloid progenitor cell development indirectly by stimulating the production of GM-CSF by macrophages (A. Billiau, Leuven). So part of the effects of treatment with interleukin-1 *in vivo* may actually mimic the effects of treatment with GM-CSF. Similarly, interleukin-2 receptors normally associated with T lymphocytes are found on many other cell types that also can respond to interleukin-2. Also, tumour necrosis factor, which synergizes with γ -interferon, stimulates interleukin-1 production and can activate the phagocytic activity of neutrophils (M. Palladino, San Francisco). A whole series of events, probably involving the generation of other cytokine messenger systems, obviously can be elicited following administration of a single cytokine. The problem is now to define some of these networks and to determine how the cytokines can be used most efficiently to promote the desired effect. This will not preclude further clinical studies provided that the situation is justified — but it will not be surprising if some of the effects seen are not quite as predicted from the assay systems *in vitro*. □

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*International Workshop on Clinical Application of Lymphokines and Cytokines, Erlangen, FRG: 5–8 March 1986.

Physiology

The expanding physiological roles of atrial natriuretic factor

from Philip Needleman

In 1981, A. de Bold and colleagues demonstrated profound excretion of sodium and water into the urine following injection of a cardiac atrial extract into rats. With the lightning pace of modern biomedical research it took just two years to describe the structure of a unique cardiac peptide and we are now in the position to begin clinical trials of a synthetic product. A recent meeting* was the first to bring together the investigators establishing the synthesis, processing, release, function and potential therapeutic use of the atrial peptide, especially the homeostatic regulation of fluid, salt and blood pressure. The start of clinical trials only five years after the first description of the activity from rat heart extract is a remarkable demonstration of the pace of modern biology.

Considerable effort has focused on the storage and processing of the hormone within cardiac tissue, and identification of the circulating form. The natriuretic vasodilator (variously termed atriopeptin, cardionatin, auriculin, atrial natriuretic factor or peptide) is stored exclusively as a 126-amino-acid precursor in granules located in both the right and the left mammalian cardiac atrium (de Bold, Ontario; G. Thibault, Clinical Research Institute of Montreal, CRIM). Cultured fetal atrial myocytes store and release the intact prohormone into the medium (J. Seidman, Harvard). Blood levels of atrial peptide are elevated by intravenous volume overloading, vasoconstrictor agents, head-out water immersion (P. Needleman), morphine (J. Gutkowska, CRIM) and salt loading. There is a direct linear correlation between the elevated plasma atriopeptin levels stimulated by vasopressin in rats and the loss of atriopeptin precursor for the right, but not the left, atrium (Needleman). The mature 28 amino-acid carboxy terminus of the rat prohormone is the circulating form (Needleman Thibault) simultaneously accompanied by a high molecular mass amino-terminal fragment of the prohormone lacking the carboxy-terminal atriopeptin (Needleman). The intact prohormone has not been found in the blood of normal experimental animals or man, suggesting that the cleavage of the precursor that produces the mature hormone occurs at the time of release from the atrial granule. The cleavage enzyme responsible is a potential regulatory step and its cellular

site and characteristics are one focus of current attention.

Immunocytochemical experiments confirm the presence of atriopeptin-like materials in the brain, especially the hypothalamus (D.M. Jacobowitz, National Institutes of Health). Chromatographic analysis of brain extracts show low levels of a peptide that co-migrates with atriopeptin-28 (the carboxy-terminus of the prohormone) (H. Imura, Kyoto; T. Inagami, Vanderbilt University). However, neuronal synthesis of atriopeptin or isolation and description of the messenger RNA for atriopeptin in brain has not been found. Autoradiographic experiments demonstrate the existence of specific, high-affinity receptors for atriopeptin in the brain (R. Quirion, CRIM) and incubation of isolated brain regions with the peptide gives elevated cyclic GMP pro-

duction (Inagami), but there are only hints of cerebral functions for atriopeptin. Systemic injection of the peptide partially suppresses the drinking response and blood pressure elevation in rats produced by angiotensin (Inagami; Imura); the salt appetite and total fluid intake in spontaneous hypertensive rats (Imura); and vasopressin release (W.K. Samson, University of Texas). These experiments imply that atriopeptin has a central role in regulating body salt and fluid levels and peripheral blood pressure.

The availability of synthetic peptides rapidly led to investigation of receptor binding and analysis of the potential second messenger for atriopeptin. Down-regulation of atriopeptin receptor density is induced by pre-exposure to atriopeptin of cultured arterial smooth muscle cells or in blood vessels isolated from rats that were salt loaded and/or treated with mineralocorticoid (T. Schiffrin, CRIM). In fact, deoxycorticosterone acetate-salt hypertensive rats showed diminished sensitivity to the hypotensive effects of atriopeptin. The current best candidate for a second messenger appears to be cyclic GMP as stimulation of particulate guany-

25 years ago

Discovery of messenger RNA

AN UNSTABLE INTERMEDIATE CARRYING INFORMATION FROM GENES TO RIBOSOMES FOR PROTEIN SYNTHESIS

By DR. S. BRENNER

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A LARGE amount of evidence suggests that genetic information for protein structure is encoded in deoxyribonucleic acid (DNA) while the actual assembling of amino-acids into proteins occurs in cytoplasmic ribonucleoprotein particles called ribosomes. The fact that proteins are not synthesized directly on genes demands the existence of an

RNA is not the intermediate carrier of information from gene to protein, but rather that ribosomes are non-specialized structures which receive genetic information from the gene in the form of an unstable intermediate or 'messenger'. We present here the results of experiments on phage-infected bacteria which give direct support to this hypothesis.

UNSTABLE RIBONUCLEIC ACID REVEALED BY PULSE LABELLING OF *ESCHERICHIA COLI*

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WHEN *Escherichia coli* cells are infected with T even bacteriophage particles, synthesis of host proteins stops¹, and much if not all new protein synthesis is phage specific². This system thus provides an ideal model for observing the synthesis of new proteins following the introduction of specific

During these years, evidence³ accumulated that the sites of much, if not all, protein synthesis are the ribosomal particles, and it was thought most likely that ribosomal RNA was genetically specific, with each ribosome possessing a base sequence which coded for a specific amino-acid sequence (one ribo-

* First International Symposium of Atrial Natriuretic Factor, Montreal, 10 - 11 March 1986, organized by M. Cantin.

cyclase is the first post-receptor event after interaction of atriopeptin with cultured cells, isolated tissue, intact animals and tissue membranes. After solubilization and extensive processing of rat lung membranes, atriopeptin receptor copurified with guanylate cyclase (T. Kuno, Stanford, Palo Alto). In patients, new evidence suggests sustained elevation of plasma cyclic GMP after bolus administration of atriopeptin, long after the labile peptide is detectable (P. Hamlet, CRIM). Furthermore, atrial peptide infusion results in higher plasma levels of cyclic GMP in essential hypertensive than in normal controls (Hamlet). Changing plasma or urinary cyclic GMP levels of patients in response to graded doses of atriopeptin could provide a useful measure of changes in sensitivity in various pathophysiological states.

In the kidney atriopeptin appears to act at different sites and by different mechanisms to those described for known diuretics or vasodilators (E. Blaine, Merck, West Point, New Jersey) but the actual mechanism of natriuresis remains unknown. Specific renal atriopeptin-binding sites have been found in the glomerulus and in the inner medulla-papilla especially on medullary collecting duct cells (Needleman; M. Cantin, CRIM). This receptor distribution is quite distinct from that described for angiotensin (Cantin) or any known diuretics. There is still no direct evidence for an effect of atriopeptin on tubular sodium transport.

The action of the peptide on glomerular filtration rate and renal blood flow is greatly enhanced by volume expansion or by elevated renal vascular tone (Blaine; Burnett, Mayo Clinic, Rochester), suggesting that atriopeptin could exert more profound effects in pathological rather than physiological situations. Natriuresis may result from haemodynamic effects that modulate sodium excretion in the distal nephron. H. Sonnenberg (University of Toronto) made the interesting suggestion that atriopeptin mediates a passive permeability change in the medullary part of the nephron allowing sodium to flow back from the interstitium into the lumen and thereby produce a large natriuretic response. The atrial peptides also exert a non-renal effect by interaction with several other hormonal systems involved in fluid-electrolyte and blood pressure regulation. Thus, atriopeptin acts as a negative modulator of aldosterone secretion (P. Mulrow, University of Toledo; T. Goodfriend, University of Wisconsin), renin secretion (M. Naruse, Tokyo; S. Atlas, Cornell), vasopressin release (Samson) and catecholamine release (O. Kuchel, CRIM).

The development of sensitive assays for atriopeptin has permitted the analysis of changing plasma hormone levels during manipulation of fluid and salt concentra-

tions and in pathophysiological states in experimental animals and human subjects. Elevated plasma atriopeptin levels are found in experimental animals with congenital hypertension (R. Garcia, CRIM), cardiomyopathy (Cantin), salt loading or water immersion (Needleman). There are elevated plasma levels in patients with chronic renal failure before dialysis (T. Yandle, Princess Margaret Hospital, Christchurch, New Zealand; I. Tikkanen, Helsinki University), high-salt diet (Yandle; D. Robertson and Inagami, Vanderbilt University), head-out water immersion (Atlas; F.R. Buhler, Basel), cardiac pacing (Buhler) or supraventricular tachycardia or exercise (Tikkanen), persistent ductus arteriosus (Tikkanen) and, finally, ischemic heart disease. The elevated levels are directly correlated with the severity of the congestive heart failure (K. Nakao and Imura, Kyoto; M. Bourassa and J. Gutkowska, CRIM). In patients undergoing cardiac catheterization for angina the highest concentration of atriopeptin is present in the coronary sinus blood (Robertson and Inagami; Nakao and Imura; Bourassa) as the carboxy-terminal peptide of low relative molecular mass (Nakao; Yandle).

Once the structure of atriopeptin was known adequate supplies of synthetic peptides were soon available for qualitative and quantitative testing in laboratory animals to establish their safety, dosage and potential use. Preliminary clinical testing of the atrial peptide has begun in New Zealand, Japan, Switzerland and the United States. The initial reports, mostly

from normal volunteers, are in good agreement with experimental animal studies. As expected, bolus injections of the peptide are more difficult to manage than infusions. Bolus atriopeptin administration produces an acute hypotensive response, sudden bradycardia, paleness and nausea in normal patients (P. Larochelle, CRIM). Constant infusion of atrial peptide into normal patients on a low sodium diet is largely without effect whereas patients on a high sodium diet respond with increased sodium and volume of excretion (Yandle; M. Nakazato, Miyazaki Medical College, Japan). Infusion of atriopeptin into hypertensive patients result in a greater increase in diuresis, natriuresis and cyclic GMP excretion than in normal control patients (Larochelle). The anti-hypertensive potential is shown by dose-dependent increases in forearm and skin blood flow following infusion of the peptide into normal subjects (Buhler; H.R. Brunner, Lausanne). An unexplained response noted in experimental animals and man is the ability of atriopeptin to increase the cellular component of the haematocrit (Brunner) suggesting that the peptide regulates volume distribution by shifting fluid between the extra- and intra-vascular compartment. Given the potential use of the peptide it is not surprising that multi-centre clinical trials are planned or started by several drug companies. □

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Transducing proteins

Chemotaxis gene unveiled

from H.C. Berg

RECENT work on chemotaxis in bacteria (*Escherichia coli* and *Salmonella typhimurium*) has focused on transducers, a set of membrane proteins that process information about changes in concentration of various sugars and amino acids. Cells lacking any one of these proteins are defective in taxis for ribose and galactose (termed *trg* mutants); in taxis for serine and certain repellents (*tsr* mutants); or in taxis for aspartate and certain other repellents (*tar* mutants). *Trg* and *tsr* are not located near any other chemotaxis-related genes on the *E. coli* genetic map, but *tar* is the first gene to be found in an operon that also consists of four other genes whose products are required for responses to all chemical stimuli. When the operon was physically mapped, another gene was found immediately downstream from *tar* that proved to be homologous with *trg*, *tsr* and *tar*. Because it was presumed to specify a taxis-associated protein, this gene was called

*tap*¹⁻³. Surprisingly, its function has remained obscure⁴. Now the puzzle is solved — as revealed by Manson *et al.* on page 253 of this issue⁵, *tap* (with acronymic fortuity) mediates taxis for peptides.

Transducers have an interesting history. Originally, Adler and his colleagues called them signallers because they appeared to relay signals from chemoreceptors, such as the ribose and galactose periplasmic binding proteins, to the flagella^{6,7}. Later, Adler's group discovered membrane proteins of relative molecular mass (*M_r*) ~ 60,000 that were methylated during adaptation to large step stimuli⁸. These proteins turned out to be identical to the signallers, namely the *trg*, *tsr* and *tar* gene products⁹⁻¹¹, and the signallers therefore became known as methyl-accepting chemotaxis proteins. Finally, it was found that Tsr and Tar proteins bound serine or aspartate directly¹²⁻¹⁴; that is, that they were receptors as well as signallers and

methyl-accepting chemotaxis proteins. As these components are so intimately involved in sensory transduction — they receive information from the external environment, move this information across the cytoplasmic membrane, generate signals that converge on the flagellar motors, and change their signalling capacity in a manner that allows for adaptation — the term transducer seems more appropriate. To learn more about these remarkable molecules, turn to page 253.

In brief, Manson *et al.*⁵ find that the *tap* gene product mediates taxis towards several dipeptides, particularly those containing L-amino acids, but to few tripeptides. The authors observe maximal responses in tethered cells with changes in concentration as small as 1 μ M. In addition, they prove that the relevant chemoreceptor is a *M*, 49,000 periplasmic dipeptide-binding protein. Finally, they speculate that synthetic peptide antibiotics could be made to which bacteria would be suicidally attracted.

The plot is thickened by the recent discovery in *S. typhimurium* of another transducer-like gene called *tip*¹⁵. Its locus is not tandem to *tar*, nor is its ligand specificity known. Manson *et al.*⁵ do not find dipeptide chemotaxis in *Salmonella*, but their strain could have been *tip*⁺. So it remains to be seen: is *tip* *tap*?

Why did taxis for peptides fall through the net so widely cast by Adler and his colleagues in their early surveys of chemotactic sensitivity? It is possible that the wild-type cells used in their studies contained little, if any, dipeptide-binding protein. Again, it might be that one man's wild type is another's mutant. □

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Explosive cyclogenesis

Large-scale circulation changes

from B.J. Hoskins

Two meteorological phenomena that are of universal interest are the rapid development of a cyclone, or low-pressure system; and the development and maintenance of a so-called blocking high-pressure system. The former can frequently be missed or underestimated by the forecaster, and the latter is associated with weather conditions lacking the usual variability. A recent paper by S.J. Collucci (*J. atmos. Sci.* **42**, 2701; 1986) attempts to link these two phenomena by exploring the possibility that explosive cyclone development initiates an atmospheric block. Collucci concludes that the nature and origin of the block is strongly influenced by the relative phases of the cyclogenesis and the larger scale flow in which it is embedded.

In the early 1940s, when charts of the atmospheric flow above the surface became available, it was clear that the normal movement of surface mid-latitude weather systems followed by the general westerly wind but that, on occasion, this flow was seemingly blocked, the weather systems being forced far to the north and south. R. Berggren *et al.* (*Tellus* **1**, 14; 1949) first described the possible importance of the feedback of the weather systems onto the block. The interest in blocking systems has not waned since then, but a complete description of their physical basis has eluded meteorologists. Because the characteristics of seasons dominated by blocking events are totally different from those that are not, there can be no confidence in forecasts of the possible climatic impacts of, for example, the increase in carbon dioxide in the atmosphere caused by the burning of fossil fuels, until we can be sure that such events are faithfully modelled.

One of the triumphs of present-day meteorology is the production of six-day forecast maps that are as good, on average, as the two-day maps of twenty years ago. Sometimes, when the weather is in a stable regime such as a block, the predictability of the atmosphere is quite a lot longer than six days. At other times, in particular when there is a sequence of depressions, the predictability of the weather at any particular instant is poor but the predictability of the regime may still be good.

The importance of the turbulence of the day-to-day weather in determining the behaviour of the seasonal or regime average flow has been a subject of such discussion. Most work using seasonal mean budgets suggests that the terms associated with transient motion are small and predominantly in the sense of damp-

ing the mean motion. But studies of individual blocking regimes, such as that by L. Illari (*J. atmos. Sci.* **41**, 3518; 1984), have tended to assign to the transients a more fundamental role in the maintenance of the regime. Modelling work has also supported the notion of a positive feedback of the synoptic timescale motions onto structures in the longer timescale planetary space-scale motion.

The modelling study of B. Reinhold and R. Pierrehumbert (*Mon. Weath. Rev.* **110**, 1105; 1982) was important in suggesting a dual role for synoptic timescale turbulence in a relatively simple system in which multiple equilibria were possible. Most of the depressions were the slaves of the larger scale motion, feeding back positively on the ambient structure to ensure the stability of a weather regime. But occasionally one of them was large enough to knock the system into a new regime. In terms of predictability, the slave depressions are helpful, but the large 'rogues' are catastrophic unless their rapid development can be understood and forecast.

F. Sanders and J. Gyakum (*Mon. Weath. Rev.* **108**, 1589; 1980) studied these latter systems, referring to them as bombs, and the present work by Collucci is aimed at futhering our understanding of the importance of these systems in changing the large-scale circulation. Collucci investigates two cases in some detail using, in particular, equations that diagnose the behaviour of the rates of changes of pressure (height tendency) and of vertical motion. In that Collucci has seemingly not used the correct thermal tendency boundary condition for the former, there is some question concerning the details of some of his results. But he presents intriguing evidence that explosive cyclogenesis affected the large-scale flow in a different manner in both cases because of its different phase relationship with that flow. In the first case, the depression developed to the east of a major trough and at the same time a weak downstream blocking anticyclone intensified and moved north-westwards. In the second, the depression developed on the eastern flank of a major ridge and led to a blocking cyclonic vortex, the blocking anticyclone occurring upstream. In each example, the weather regime for the next month over at least a quarter of the hemisphere seemed to be strongly influenced by the explosive cyclogenesis. □

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Gene expression

Negative regulation of enhancers

from Nicholas C. Jones

THE molecular events that underlie the process of cellular differentiation remain obscure, but it is widely assumed that at least some of them will closely resemble those that accompany the neoplastic transformation of cells. One possible link between transformation and differentiation involves the gene that encodes the E1A early antigen of human adenoviruses which, together with the viral gene encoding E1B, is responsible for the transformation of primary rat cells by the virus. One role of E1A in transformation is to establish the primary cells so that they can grow indefinitely *in vitro*. The E1A protein is also known to be a transcriptional regulator and can activate or repress the transcription from various promoters (DNA elements that initiate transcription). It now seems that in undifferentiated embryonal carcinoma (EC) cells there exists a regulatory activity, lost on differentiation, that very closely resembles that of E1A. Therefore, although there is no cellular counterpart of the gene encoding E1A as judged by DNA sequence homology, there does seem to be a functional counterpart. The similarity between E1A and the EC regulatory activity is strengthened by a report from Pierre Chambon's group on page 249 of this issue¹, which demonstrates that both types of activity repress expression of a gene by acting through the same target DNA sequence.

Repression

Repression by the E1A protein(s) involves enhancers, regulatory *cis*-acting DNA elements found upstream or downstream of several viral and cellular promoters which stimulate initiation of transcription from these promoters². Enhancers can do this in an orientation- and relatively position-independent manner, although the mechanism by which they stimulate transcription is unknown. Chambon and his colleagues were the first to show that some enhancers can be negatively regulated by products of the E1A gene, including the polyoma virus and simian virus 40 early gene enhancers³ and those that control the expression of the genes encoding the immunoglobulin γ_{2b} heavy chains and κ light chains⁴.

This result was a surprise because the same protein is known to activate transcription from many promoters, including those associated with the adenoviral early genes^{5,6} and the human major heat shock gene⁷. Many of the same enhancers that are repressed by E1A are also unable to function efficiently in undifferentiated EC cells. One intriguing possibility, there-

fore, is that the cellular factor that represses enhancers is related to E1A. A previous observation made by Nevins and his colleagues⁸ suggests that this is likely. Adenovirus mutants defective in the E1A gene fail to replicate in differentiated cells because the viral early genes that depend on E1A activation are poorly expressed. In contrast, EC cells can support growth of such mutants, which implies that these cells contain an E1A-like activity, enabling transcription from the early genes to take place even in the absence of E1A. Therefore, both the transcription-activating and repressing activities of E1A are mimicked by EC cells and, by analogy to E1A, it is possible that both activities are caused by the same gene product.

Negative regulation of enhancers helps to explain the inability of certain viruses, such as the polyoma virus, to replicate in undifferentiated cells. Although wild-type polyoma virus fails to replicate in EC cells, several mutants have been isolated and characterized that can replicate; these mutants contain altered enhancer sequences that activate transcription from the polyoma virus early gene equally well both in differentiated and in undifferentiated cells^{9,10}. If, as suggested above, the cellular activity that represses enhancers and E1A both act by the same mechanism and through the same target sequence then the enhancers present in the polyoma virus mutants should not be repressed by the E1A protein. The new work by Chambon's group¹ shows that this is indeed the case.

The authors studied the expression of a cloned rabbit β -globin gene that was linked either to the polyoma virus wild-type enhancer or to a mutated enhancer present in the polyoma virus mutant ECF9-1 that can efficiently replicate in EC cells. They measured expression of these genes in mouse L-cells that did or did not contain a stably integrated copy of the E1A transcriptional unit. In the cell line that does not express the E1A gene, both enhancers efficiently stimulate transcription of the β -globin gene, but in the presence of E1A, activity of the wild-type polyoma virus enhancer is repressed at least fivefold. In contrast, the mutated polyoma virus enhancer is unaffected by E1A and stimulates transcription of the β -globin gene equally well in both cell lines. Therefore, the authors conclude that the mutated enhancer is resistant to repression by E1A, which strengthens the argument that both E1A and the activity in undifferentiated cells repress enhancers in a similar fashion.

The physiological role of the cellular E1A-like activity is unknown but could be fundamental in regulating cell differentiation. Differentiation is accompanied by many changes in gene expression, some of which may be a consequence of losing the E1A-like activity. The E1A-like activity could contribute to the maintenance of the undifferentiated state of the cell by activating the expression of genes that play a crucial part in growth and division of such cells; and at the same time repress the expression of genes which are required to be active only in the differentiated state. If the stimulus for differentiation somehow results in a reduction in this activity, then the pattern of expression of these two sets of genes will be reversed. Whether the loss of the activity is merely a consequence or whether it is the cause of differentiation remains to be seen. It goes without saying that recognizing and cloning the gene that encodes this activity would be greatly significant, although such a feat would be far from trivial. It would also be of interest to identify cellular genes that are activated or repressed by E1A as these genes could normally be targets of the cellular E1A-like product.

Establishment

The relevance of the cellular E1A activity in differentiation may be better understood when the role of the adenovirus E1A gene itself in primary cell establishment is elucidated. What is the importance of the regulatory activities of E1A to establishment? Isolation and characterization of E1A mutants will be helpful in answering this question. Can mutants be isolated that have altered regulatory activities but still transform, and vice versa? The answer seems to be yes. At early times of infection or in cells transformed



Two species of ibises (Plataleidae) have been recognized in the early Pliocene avifauna from Langebaanweg, south-western Cape Province, South Africa. The figure shows the partial skeleton (A) of *Geronticus apex* and represents the first Tertiary record of this genus. B, *G. eremita*; C, *G. calvus*. Scale in mm. From *Ann. S. Afr. Mus.* 97(3), 57 (1985).

by adenovirus, two different messenger RNA molecules, 12S and 13S in size, are synthesized from the E1A gene. In common with wild-type virus, E1A mutants that can only synthesize the 12S messenger RNA can still collaborate with the *ras* oncogene to facilitate morphological transformation of primary cells and can repress the activity of enhancers. Unlike wild type, however, they do not efficiently *trans*-activate the viral early promoters^{11,12}. Conversely, several mutants have been isolated that can *trans*-activate as efficiently as the normal E1A gene but do not collaborate with *ras* to effect morphological transformation (J. Schneider and my own unpublished observations). So it appears that at least the *trans*-activating and transformation activities of E1A can be separated. It remains to be seen whether these mutants can still repress enhancers, as the possibility still

exists that it is the repressing activity of E1A that is crucial for its role in transformation. □

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Neurobiology

Pigment and visual projections

from R.D. Lund

TWENTY years ago it was first noticed that in albino rats the optic axons make abnormal connections in the brain¹. Subsequent work showed that this phenomenon is widespread in animals with reduced eye pigmentation, including Siamese cats and man (reviewed in refs 2,3). The disorder is of special interest because it bears on the fundamental problem of the central representation of visual space. The cause of the abnormal projections in albinos is still a mystery but new studies⁴⁻⁶ suggest that there may be a relationship between lack of pigmentation and incorrect timing of axon outgrowth.

There are basically two strategies of mapping the visual world on the brain in vertebrates — retinal and visual field mapping. Either the image seen by the whole retina of each eye is projected to the opposite side of the brain, or the contralateral visual field, seen by part of the retina of each eye, is mapped on each brain half. The first pattern is typical of the retinotectal pathway of lower vertebrates, such as fish and frogs. Axons from one eye must cross over those from the other in the optic chiasm on the ventral side of the brain (*a* in figure). The second pattern, found in mammals in the pathway from the retina to the lateral geniculate nucleus results from a more complex sorting of axons at the optic chiasm (*b* in figure). Axons from ganglion cells located more nasally in the retina cross in the optic chiasm, but those originating from more temporal cells innervate brain regions ipsilaterally to their origin and do not cross the midline. The line on the retina defining whether cells send axons contralaterally

or ipsilaterally corresponds to the representation of the vertical meridian, which receives images projected directly in front of the animal.

Reduced to its simplest, the anomalous projection pattern in hypopigmented animals represents a confusion of the two strategies — there is both visual field and retinal mapping in an animal where normally only the visual field is mapped. How the presence of pigment and patterns of axonal distribution interrelate is unknown. It is clear that pigmentation of the epithelium lying adjacent both to the outer surface of the neural retina and to

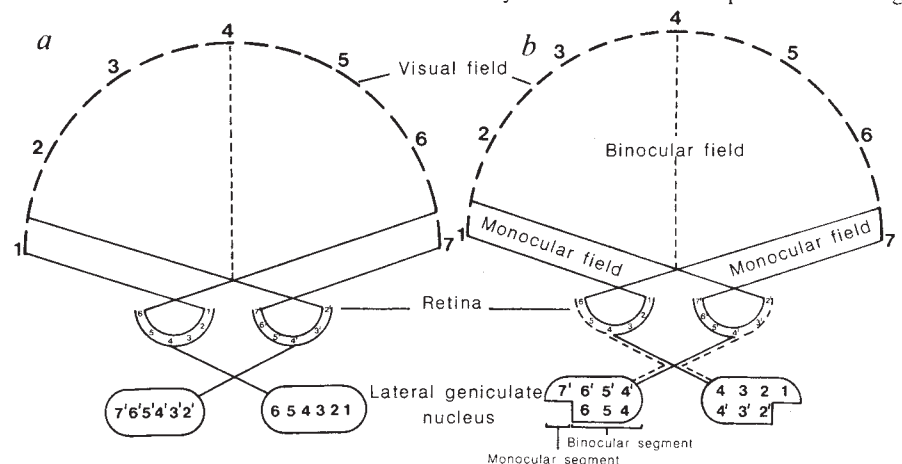
the axons of the optic nerve is particularly important.

A view put forward by several investigators^{2,4,7} directs attention to the transient pigmentation of the optic stalk, suggesting that the pigment limits the optic axons to a narrower and more orderly channel as they approach the optic chiasm. Absence of pigment may result in inappropriately ordered axons.

A second view places the emphasis on differential timing of axonal outgrowth; Dräger's results⁵ suggest how a pigment defect could lead to a timing disorder. She finds that pigmented cells are highly efficient at binding calcium ions. This may normally reduce the level of calcium in adjacent retinal cells and influence the timing of closure of gap junctions. In turn, this might alter intercellular communication patterns, so regulating the timing of overall maturation of the retina. In hypopigmented mutants, calcium binding levels are low, which may substantially alter the timing of maturation.

The misrouting of optic axons presents many problems in the distribution of terminals in the central visual nuclei, such as the lateral geniculate nucleus, and in the relay to the visual cortex. Guillery and his coworkers², who previously investigated these pathways in the Siamese cat, have now looked at them in the ferret⁸. The ferret is suited for the study of the development of the visual system because the visual centres are both highly differentiated as they are in cats and have the advantage of being immature at birth.

In albino ferrets with the eyes intact, the representation of the geniculate on the cortex conforms to one of two patterns⁹: either the main aberration is an accommodation of the enlarged crossed projection to fit a normal visual-field map; or it may be a more bizarre pattern resulting



a, Retinal mapping. Plan of the distribution of the visual image when each retina projects to the contralateral side of the brain. *b*, Visual-field mapping. Plan of the representation of the visual image on the retina and the distribution of axons in the optic chiasm and brain. Each nasal retina projects to the contralateral hemisphere, whereas the temporal retina projects ipsilaterally, ensuring a representation of the contralateral visual field on each side of the brain. In hypopigmented mutants, there is a bias towards pattern *a* in situations where pattern *b* would be expected. Additional crossed axons (3', 2' and 6, 5) may be mapped, although they sometimes map as if matching appropriate field positions but on the wrong side of the brain.

from a partial attempt to map a whole retina on one cortex. There are also individual cells in the lateral geniculate nucleus that project on the cortex with no obvious pattern. If the eyes are removed at a sufficiently early stage of development, before they innervate the lateral geniculate nucleus, the map of the geniculate on the visual cortex is normal⁶. Thus, any disorder in the geniculo-cortical projection is secondary to the retinal defect and is not a direct result of the mutation.

Such studies suggest that the ferret system is ideal for unravelling the developmental patterns underlying the two proposed strategies for projecting the visual field on the brain, and should provide new insights into the mechanisms of interdependence in developing neural path-

ways, as well as the establishment of topographical representations in different regions of the brain. The recent work shows promise for defining the mechanisms involved and may help to elucidate the genetic control of a component of neural development in vertebrates. □

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Palaeoecology

Unravelling human effects

from Peter D. Moore

WHEN the early pollen analysts began to describe the pattern of Britain's vegetation following the last retreat of the glaciers, change in climate was seen as a major factor. The gradually rising temperature peaked some 7,000–5,000 years ago and has subsequently undergone a somewhat erratic decline. This was the basis of their climatic model and major vegetation changes, as reflected in fossil pollen spectra, were considered within this framework. The alder (*Alnus glutinosa*), a species showing postglacial expansion, has been regarded as a marker of changing climate, but new evidence¹ strengthens the claim that mesolithic man may have been involved in its spread².

Only one species of alder, *A. glutinosa*, has been recorded in the British postglacial period and the expansion of its pollen, coincident with the decline of pine, was used by Godwin³ to mark the commencement of the Atlantic period (zone VIIa) of the Holocene in which the climate reached its optimum. Scattered *A. glutinosa* pollen was found in earlier postglacial deposits but was considered by Godwin¹ to represent local populations of the tree surviving in sub-optimal conditions. The expansion of this species was regarded as a consequence of increased Atlantic wetness rather than to increasing temperature. Thus the increasing presence of alder became an established datum which was assumed to be reasonably synchronous through England and Wales: it became synonymous with the Boreal–Atlantic transition.

Its value as a means of dating sediments was called into question by Oldfield and Statham⁴ in the Lake District (north-west England), and soon afterwards Smith⁵ pointed out that the changes necessary for

alder to become more abundant within a forest and pine less so must ecologically be very complex. The climatic threshold of wetness, which was thought to induce this change, would have varied with geographical setting, and the forest itself would possess a degree of inertia resisting such a change, but the essentially climatic basis of the event was not questioned.

The expansion of alder with time was finally revealed by Smith and Pilcher's⁷ collation of radiocarbon dates from various parts of the British Isles. The rise in alder varied between 7,500 and 5,000 years ago in different parts of Britain, but there was no strong geographical trend, except perhaps a tendency for earlier increases at lowland sites. The actual pattern of its expansion across Europe has been mapped by Huntley and Birks⁸, whose pollen maps show that the tree was undoubtedly present in the west of France some 10,000 years ago, but its abundance there seemed to decline over the following 2,000 years. Where the British population arrived from and where it first entered the islands is unclear, but the pollen maps do suggest that the tree came westwards across northern Europe and entered Britain from the south-east, possibly along the Thames estuary through the early Holocene swampland.

But this proposed pattern is now considerably upset by the discoveries of Chambers and Price¹ in the peats of a valley mire in north-west Wales. They identify a very early alder expansion about 8,500 years ago. This follows suggestions already made by Chambers⁹ that the alder spread into Wales from the north-west, arriving later in the south. But even this pattern is an oversimplification, because at least one site in South Wales has re-

vealed pollen dated to 7,900 years ago at Llangorse Lake¹⁰. These findings support an alternative idea that there was a source of alder to the west of Britain in the late or early postglacial, perhaps in lands now covered by the Irish Sea. But what was the actual cause of the population expansion of alder at these sites?

Smith² suggests the sudden success of the species results from the disturbance of forest inertia by mesolithic man. This possibility was actually suggested first by McVean¹¹ in 1956; his ecological studies on alder showed it to be tolerant of competition and an efficient colonist, but he had no data supporting his idea of a human link. Nearly 20 years later, Smith reports evidence in South Wales of forest destruction associated with a rise in alder pollen, particularly the presence of charcoal in those horizons where the pollen becomes more abundant. There are similar records of charcoal at the site of Chambers and Price¹.

Mesolithic peoples possessed a great potential for modifying their environment by the use of fire, to an extent that has only recently been realized¹². There are several ways in which alder could benefit from such activities. Disturbance and burning of catchments leads to increased runoff of water, causing greater erosion and nutrient flushing in the basins, all of which would favour alder. The burning of reed swamp itself leads to the growth of shorter (but denser) and less robust reed shoots¹³, which could supply alder with opportunities for invasion. Also, the felling of alder leads to vegetative sprouting and cloning¹⁴ which could result in its rapid spread in swamp forests.

The decline in elm some 5,000 years ago; the decrease in lime somewhat more recently; and the spread of heathland, moorland and blanket bog are all now regarded as intimately linked with, if not actually caused by, the activities of prehistoric human activity. It now appears that the postglacial expansion of alder may be a consequence of its ability to benefit from human activities. □

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Novel freedom of expression

Loren Graham

Soviet Fiction Since Stalin: Science, Politics and Literature. By Rosalind J. Marsh. Croom Helm/Barnes & Noble: 1986. Pp.338. £25, \$28.50.

DOES the Soviet Union suffer from the same grievous split between the scientific and literary cultures so graphically described many years ago by C.P. Snow in the Rede Lecture at Cambridge? Some evidence suggests that it does not, that scientists and creative artists have more in common in the Soviet Union than they do in most Western countries. Distinguished Soviet theoretical scientists, especially in physics and mathematics, have a tradition of defence of freedom for creativity in general. For example, in the waning years of Stalinism physicists such as Peter Kapitsa and Igor Tamm created and protected salons celebrating the arts; even today, over a year after Kapitsa's death, the walls of his famous Institute of Physical Problems in Moscow are lined with paintings that cannot be exhibited elsewhere in the Soviet Union.

Yet there is another split in the educated élite of the Soviet Union, one whose two opposing sides were defined some years ago by the West German scholar Arnold Buchholz as the "scientific-literary intelligentsia" and the "techno-managerial intelligentsia". The first group takes as its primary goal "creativity", in whatever field — be it physics, poetry or art — and is united by opposition to the ideologists and censors of the regime. The second group has as its main aim the management of society and the economy; its members have in common a determination to defend and improve the existing Soviet order. Members of the first group often consider those in the second group to be philistine and dogmatic; those in the second group just as frequently consider the first group irresponsible and unpatriotic.

In recent years specialists on the Soviet Union have begun to debate this issue, but Rosalind Marsh, a lecturer in Slavonic Studies at Queen's University, Belfast, is the first person to convert debate into scholarship. In this book she has succeeded marvellously in laying bare the image of science and technology in Soviet fiction, and in the process she has enriched our knowledge both of the Soviet Union and of the relationship between the world of scientists and the world of artists. Her book places our understanding of the relationship between the two cultures in the Soviet Union on an entirely different and more sophisticated plane.

Marsh observes that many of the characteristics of the genius seeking truth

(individualism, creativity, spiritual freedom) that are attributed to scientists in Soviet fiction are associated with artists in the West. She suggests that the reason for this difference is that such an artist would be too controversial in the Soviet Union in view of the Communist Party's continuing ideological supervision of art. Science, however, has in the post-Stalin years escaped from most of its ideological restrictions, and therefore can serve as a model for free creativity.

Science is admired and praised by both the Soviet regime and the non-conformist intellectuals, although for slightly different reasons: the regime wishes to benefit

from the practical applications of science for the economy and for national strength, while the non-conformist intellectuals yearn for free debate of social and political issues. Fiction involving scientists becomes for the non-conformist a surrogate for broader debates that are not allowed in a more literal form. As Marsh says, "Through the mouth of a character or by implication, writers are often able to suggest ideas which they could not express in their own names".

In the post-Stalin period, Soviet fiction dealing with science and technology has been the medium for raising a host of questions that are not permitted elsewhere. Is the space programme so expensive that it impoverishes the domestic economy? Has the collective farm system destroyed the peasants' pride in their work and love of the land? Is the centralized Soviet economy a drag on technological innovation? Does mind-numbing work on assembly lines alienate workers in the Soviet Union just as it does in the West? Is the emphasis on rapid industrialization polluting the Soviet environment? Is urbanization eroding traditional life and culture? And even: Are the causes of the threat of nuclear destruction not solely to be found in the West, but shared by the superpowers? Marsh explores all of these themes and many more, showing how science and technology in fiction is one of the most revealing mirrors available to us for viewing controversies in Soviet society. She makes a convincing case that scientists and engineers in novels are permitted greater latitude in their discussions of social issues than politicians ever would be.

An example of how a novel about technology can become an argument for reform is Ivan Dvoretzky's *The Outsider* (1972), in which the brash young engineer Cheshkov is horrified by the waste and inefficiency of a Leningrad factory and attempts, at one swoop, to eliminate laziness, corruption, truancy, drunkenness, falsification of records and outright theft of state property. At first Cheshkov causes a rebellion, and nearly loses his job, but eventually his radical reorganization of management and labour in the plant wins official support.

The prominent economist Academician A. Aganbegyan recognized that novels such as *The Outsider* are important in efforts to reform the Soviet economy. Writing in 1974, he proclaimed:

Economists now know what must be fought for. But public opinion has not sufficiently matured for many economic steps to be taken... What economics asks of literature is that literature use all its resources to prepare public opinion for changes connected with the scientific and technological revolution.

Aganbegyan has now been transferred from his old post in Novosibirsk to

History for sale

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Illustration from The Botanical Magazine, a complete set of which (184 volumes containing 10,566 plates, all but three of them in colour) was on offer at Sotheby's, London, on 9 May. The periodical first appeared in 1787 and ran until 1983 when it became part of The Kew Magazine; among its editors were the founder William Curtis and, in the nineteenth century, W.J. Hooker and J.D. Hooker.

The set (estimated price £40,000 – £50,000) failed to find a buyer.

Other properties at the sale were first editions of Charles Darwin's On the Origin of Species... (sold for £1,980), Robert Hooke's Micrographia (£2,750) and Sigmund Freud's Die Traumdeutung (£1,430).

Moscow, where he is serving as advisor to Secretary Gorbachev in the latter's attempt to rejuvenate the economy. Ironically, the move of Aganbegyan to the centre of things in Moscow means that he has inevitably lost his cachet with the more free-wheeling reformers of Novosibirsk, and is now increasingly seen as one more member of the "techno-managerial intelligentsia" serving the establishment.

Marsh is understandably much more knowledgeable about literature than she is about science. Her statement that under Stalin "...certain important subjects, such as classical genetics, the theory of relativity, quantum mechanics, resonance theory in chemistry, the 'big bang' theory in cosmology and the whole new field of cybernetics were banned altogether" is far too simple. Relativity theory and quantum mechanics never were banned in the Soviet Union, despite some philosophical in-fighting over them, and, in fact, only genetics was entirely proscribed. Soviet scientists in structural chemistry, cosmology and cybernetics were not seriously hindered, since they rather easily found ways to evade ideological restrictions by adopting different terminology.

Marsh also has a tendency to cast most issues in political terms, thereby sometimes obscuring subtle intellectual nuances, especially when they concern science. She frequently evaluates Soviet scientists on the basis of whether they support official ideology and therefore overlooks the fact that eager defenders of the regime's policies have on occasion advanced important scientific ideas (an example is the physicist Nikolai Basov, who won a Nobel Prize), or that a critic of Stalinism can still be devoted to the principle of uniting Marxism and science (an example was the outstanding physicist Vladimir Fock).

Viewed against the background of her impressive achievement, however, these flaws are minor. Rosalind Marsh has given us a map to a little-explored area of the Soviet mind, the relationship of science and literature, and all who wish to enter that area will benefit from her cartographic skill. □

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Circuits in colour

L.W. Swanson

Chemoarchitecture of the Brain. By Rudolf Nieuwenhuys. Springer-Verlag: 1985. Pp.246. Pbk DM 99.

THE nervous system is a biological computer that orchestrates metabolism, as well as behaviour and its psychological counterparts. While great strides are now being made in understanding the cellular and molecular biology of chemical synaptic transmission between individual elements within the circuitry of this computer, complementary knowledge of the organization of its functional subsystems will ultimately be necessary to explain the biological foundations of behaviour. Neuroanatomy has a long and rich tradition of interest in this problem, and Professor Nieuwenhuys has provided a thoughtful, even-handed evaluation of many of the issues involved.

The seeds of the revolution dealt with here were sown in the early 1960s with the introduction of the Falck-Hillarp method for the cellular localization of monoamines in the central nervous system (CNS), and its early exploitation and refinement by the Swedish school of histochemists. The power of this method was threefold: it formed a bridge between anatomy and biochemistry by providing information about possible neurotransmitters within specific pathways; it revealed three new neural systems; and it

suggested that these systems were unlike anything previously known, as they consist of small, discrete groups of neurones that innervate most of the CNS in a seemingly rather diffuse way. A decade later came the introduction of immunohistochemical methods. Not only did these methods provide independent support for the conclusions drawn from the histofluorescence approach, but, more significantly, in principle they allowed the cellular localization of any neurotransmitter, receptor or other antigen.

The application of histochemical techniques has generated massive amounts of information about the organization of neural circuitry, and has led to a certain hybrid vigour that has rekindled interest in neural circuit organization. This has occurred for two reasons. First, much of the early work was carried out by histochemists who worked outside the tradition of classical neuroanatomy. Second, many of the neurones that were found to contain monoamines and neuropeptides lie outside the relatively well-known systems subserving the transmission of sensory information and the production of motor responses; instead, they predominate in the least-understood parts of the brain, particularly the so-called limbic system and reticular formation.

Chemoarchitecture of the Brain is an attempt to bring together the fruit of classical research with what has been learned from a somewhat different vantage point. In this it is largely successful. To date, the topic has been approached with varying success in multi-author books and limited

review articles, but this volume is a critical review of the literature which covers most of the CNS from a uniform perspective.

The book is divided into three parts. The first consists of a series of maps showing the distribution of neurotransmitters as projected onto a schematic view of the human brain. These maps are based largely on work on animals (primarily rats), and specialists will not agree with all of the details and will raise the obvious question of possible species differences. The second part sets down a number of generalizations that may seem obvious to some, but which are documented systematically here for the first time. Some of the more intriguing points raised are that no obvious structural or functional rules have emerged to explain the pattern of neurotransmitter expression in various sites; that most regions of the brain are heterogeneous with respect to neurotransmitter content; that neurotransmitter-specific cell types often do not respect traditional neuroanatomical boundaries; and that at least some neurones contain more than one neuroactive substance.

It is the third part of the book that is the most interesting, however, because here the author speculates about the possible existence and identity of a neural system whose organization is said to emerge from the histochemical data. In brief, Nieuwenhuys suggests that regions containing a large number of the currently identified transmitters constitute a readily identifiable entity (the "[paracrine?] core of the neuraxis"), which is surrounded in the brainstem by "median and lateral paracore" adjuncts. This core stretches the length of the CNS, from the olfactory bulb through the spinal cord. Structurally it corresponds largely to the dorsal horn, reticular formation and limbic system, while functionally it is postulated to subserve mechanisms that assure survival of the individual and of the species.

It is difficult not to agree with this conclusion, which follows traditional lines of thought. The novel suggestion is that this "system" may rely heavily on "paracrine neurotransmission", that is, on the non-synaptic release of transmitters from varicosities, unmyelinated axons, as has long been known to occur in sympathetic fibres. It will be extremely difficult to prove this hypothesis, however, unless a histochemical marker for transmitter release sites can be developed.

On the whole the book provides an excellent summary of contemporary views in chemical neuroanatomy and of their impact on what has often been a very conservative discipline. It should be required reading for students of neuroanatomy, while workers in other fields will find it a helpful introduction to a most complex field of research. □

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Relations amongst the populations

Eric Sunderland

The Peoples of Southern Africa and Their Affinities. By G.T. Nurse, J.S. Weiner, and Trefor Jenkins. Clarendon:1985. Pp.409. £45, \$69.

THIS is the third volume in the Clarendon series *Research Monographs on Human Population Biology*. The earlier titles *Aboriginal Man Adapting* (by R.L. Kirk) and *The Genetics of the Jews* (by A.E. Mourant, Ada C. Kopeć and Kazimiera Dominiewska-Sobczak) were well received, and the present book merits a similarly enthusiastic reception.

In the preface, Nurse and his colleagues write:

Some previous work on the human biology of Southern Africa has tended towards the creation of false categories, by assuming sharp demarcations and convenient uniformities. We have preferred to consider the Khoisan, the Negroes and the Caucasoids, not only in their unity, but in their diversity, though the more bewildering diversity of the hybrid populations has come close to defeating us.

The authors have tried to find explanations of their own observations, as well as those of others, from their three individual perspectives — that of a physiologist/palaeoanthropologist, a human geneticist well-grounded in clinical epidemiology, and a medically-qualified anthropologist with experience of the tropics and some knowledge of linguistics. This is a formidable array of expertise which has here been deployed to excellent effect.

The book opens with a chapter devoted to the environmental context within which man existed and exists in Africa, and this is followed by a succinct account of human origins (which are essentially African) and of human evolutionary development. Thus the scene is set, and it is established that in this development migration and thus genetic interchange is inextricably involved:

[from] perhaps as far back as 35000 BP... Khoisan or Khoisanoid populations were in evidence over the... area extending from Zambia and Zimbabwe southwards through the modern republic. ... The paucity during this long period and over this large territory of any groups diagnosed with certainty as Negroes, is phylogenetically highly significant.

It would thus seem that there was a Bushman/Hottentot basal population in southern Africa, upon which Negro, European and Asian groups were superimposed by migration at various later times, and to variable extents, and with which there was a measure of hybridization.

Logically, therefore, the book describes

the biological attributes of the Khoi, the San and the Negroes (including a consideration of the incursions of the latter), and then proceeds to consider the sea-borne immigrants from various parts of the world. Finally, the various hybrid communities are considered, including the Cape "coloureds", the Griqua and the Bastards.

The nomenclature used in describing these many and diverse ethnic groups is complex, even bewildering unless one has some familiarity with the relevant literature. The tables (pp.299–346) are very helpful in identifying the various populations, though it is a pity there is no map to make things easier for the reader. However, the necessary facts about groups and their locations are painstakingly set out, and one can but be content with the detail and accuracy with which this information is presented.

In addition to description, modern biological anthropologists are very properly concerned with process — natural selection, the founder effect, random genetic drift, migration, demographic characteristics, health-status and much more besides. More generally they investigate the adaptive processes which have produced the biological constitution of the peoples concerned. Such processes — and all manner of related phenomena — are considered, explicitly or implicitly, throughout the book. There is discussion of morphology, anatomy, physiology, energy flow, pigmentation, monogenic factors, culture and technology, and ecology, to name but a few of the factors which, *in toto*, characterize, and indeed constitute, the distinctive adaptive complexes that are found among the populations of southern Africa.

The authors tell us that

There has been only negligible gene flow from Caucasoids into the Southern African Negroes in modern times, and most of the undoubtedly large genetic contribution made to them by the San and Khoi cannot be dated accurately. Much of it probably happened early.

Furthermore, while the affinities of the populations within southern Africa are considered in the book, it is conceded that "It is impossible to reconstruct the precise patterns of relationship among the African peoples". However, an attempt is made by looking at 23 sub-Saharan populations in terms of 32 alleles (five red cell antigen, two serum protein and four red cell enzyme systems), using a variant of principal components analysis known as correspondence analysis. The conclusion is that

The position in space of the various populations conforms very strikingly with the geographical distances between them, as well as with reconstructions of African pre-history which are becoming available from linguistic and archaeological data.

This is reassuring — such correspondence

New journals review



On 25 September *Nature* will publish the sixth annual review supplement devoted to science journals.

Criteria for inclusion of a journal in the 1986 issue are that:

- (i) the first number appeared, or the journal was retitled, between June 1984 and May 1985 (the second cut-off date allows at least three issues of a journal to have been published, the minimum number on which a reasonable judgement can be based);
- (ii) it is published at least three times a year;
- (iii) the main language used is English.

Publishers and learned societies are invited to send four different issues of each suitable periodical, including the first and most recent numbers (if from outside the United Kingdom, by air mail) to: The Review Editor, *Nature*, 4 Little Essex Street, London WC2R 3LF, England. Subscription details for both 1986 and 1987 should be included.

is not always found — and suggests an overall coherence in the data of various kinds which have been examined. The same technique was used to consider the affinities of 42 southern African populations; here the authors say that "Perhaps the most interesting point is the extreme dissimilarity of the San populations to their Negro fellow-Africans".

The book concludes with tables of genetic polymorphisms, as well as a splendid bibliography, glossary, author index and subject index. Altogether, this is an outstanding book, scholarly yet written in lucid style. It should be read not only by those who have an interest in the peoples of southern Africa, but by anyone attempting to understand the nature and extent of population diversity in any part of the world. □

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Taking the skeleton out of the cupboard

Peter Sheterline

The Cytoskeleton: An Introductory Survey. By Manfred Schliwa. Springer-Verlag:1986. Pp.326. DM 172.

A DECADE or more of enthusiastic research on the structure of the cytoplasmic compartment in eukaryotic cells has been based on the premise that therein lies the apparatus by which the motile, morphological and polarized attributes of cells are defined. From such studies has emerged the principle that these organizational activities reside primarily in the dynamic behaviour of an integrated network of protein polymers, and their interactions with other structures in the cell. There lingers, however, a divisive and loosely applied terminology where "cytosol" is used to describe aspects of the cytoplasm in a metabolic context, and "cytoskeleton" to describe the polymer systems, when neither the cytosol nor cytoskeleton are the cytoplasmic compartment. Rather, they are operational descriptions of, respectively, what remains after centrifugation of disrupted cells or after extraction of cells with buffers containing non-ionic detergents. The title of Schliwa's well-produced book perpetuates this unsatisfactory terminology, though the pedantically-minded are appeased in a short, but useful, chapter on the cytoplasm towards the end.

As its title suggests, the book is restricted largely to the morphological and biochemical properties of microtubules, actin filaments and intermediate filaments. Even so the relevant literature is enormous, and Schliwa's bias towards the properties of filaments in higher animal cells, although reflecting the emphasis of interest in the field, is not really appropriate for the intended audience of non-specialists.

In writing a book such as this, difficult choices also have to be made in coping with subject matter which ranges freely from the phenomenological to the biophysical. These problems are compounded by uncertainties over the functional significance for cell behaviour of identified proteins, and even of the filaments themselves. Schliwa has chosen to divide his material between two sections, one on components and the other on interactions. The result is a rather arbitrary

• The third volume in the series of special issues of the journal *Nucleic Acids Research*, *The Applications of Computers to Research on Nucleic Acids*, has been published by IRL Press. Editors are D. Söll and R.J. Roberts, price is pbk £35, \$63. The previous volume was reviewed in *Nature* 309, 648 (1984).

allocation of, for example, actin-binding proteins to different chapters, depending on how the author perceives their primary function. The text is thus rather bitty, which detracts from its value as an introduction to the field, as does the incomprehensible absence of any serious discussion (or illustrations) of myofibrils, the cleavage furrow, the mitotic spindle or cilia and flagella. Less serious are the large number of typographical errors and occasionally idiosyncratic prose, for which the publishers must take some responsibility.

Work up to about the last quarter of 1984 is covered, with references in an excellent bibliography at the end. Although the book's usefulness as an introduction to cytoplasmic filament systems is compromised by its omissions, it will be valued by most cell biologists and students of the cytoplasm for its conscientious coverage of the morphological, and to a lesser extent biochemical, literature. □

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Past differences

Martin Welch

The English Settlements. By J.N.L. Myres. Clarendon:1986. Pp.248. £15, \$24.95.

IT IS now 50 years since Collingwood and Myres published their classic English history textbook, not superseded until the late 1960s by Frere's *Britannia* (Routledge and Kegan Paul, 1967) for Roman Britain and Myres's own *Anglo-Saxon Pottery and the Settlement of England* (Clarendon, 1969). This time the Anglo-Saxon settlements have been treated to a separate volume in the *Oxford History of England* series. Does it match the authority of his 1936 chapters? I think not, for though Myres has made valiant efforts to catch up with the current literature, in essence he presents again the views he established in the 1950s and 1960s.

His discussions of the literary sources show him to be out of touch with recent work on source criticism undertaken by David Dumville and others. Archaeology has a more crucial role to play with so little written material, but Myres and I stand poles apart in our interpretation of the same evidence. Myres emphasizes the presence of Germans, including Saxons, in Late Roman Britain, seeing an unbroken continuity to independent Anglo-Saxon rule. In my opinion, however, there was a marked discontinuity between the collapse of the Roman economy with its material culture c. AD 400 and the subsequent foundation of Early Anglo-Saxon settlements, usually represented by their cemeteries. It seems strange to me that Myres can recognize the contrast with Gaul, where Roman towns and villa estates survived under Merovingian Frankish rule and the situation in Britain, where they did not, yet press on with what seems to me at best an ephemeral case for archaeological continuity from Roman Britain to Saxon England.

He believes that the Saxon Shore (*litus Saxonicum*), a Late Roman coastal defence chain stretching from the Wash to Portsmouth, was so named because

Saxons were settled along it by the Romans in the fourth century, if not earlier. Yet the Gothic bank (*ripa Gothica*) on the Danube was a Roman defence against the Goths, not a zone in which they were settled, so why should the Saxon Shore be different? Myres invented the concept of Romano-Saxon pottery in 1956 to support this view, but he disguises here how effectively John Gillam and William Roberts have demolished his case, though he provides references to both on pp.92 and 89 respectively. Nor does he explain that his attribution of fourth-century dates to some Anglo-Saxon pottery cremation urns is on the basis of analogies with pots of this date on the continent or in Scandinavia and is not confirmed by the presence of fourth-century Germanic metalwork in the English pots. Germanic pottery fashions were notoriously conservative and in my opinion all these urns probably belong in the fifth century, to a series of historically attested migrations across the North Sea. These migrations appear to have resulted from invitations by the British authorities to Saxons to fight for them as mercenaries against the threat of coastal invasion by the Picts of Scotland, effectively replacing a Roman army which had been withdrawn from Britain by AD 407. The Saxon mercenaries subsequently declared independence and founded settlements, which often developed into kingdoms.

Myres has an established expertise with Anglo-Saxon pottery, but his dating and discussion of Late Roman and Anglo-Saxon metalwork is often inaccurate and misleading. Thus his discussion of Quoit Brooch Style metalwork does not even mention the Mucking Grave 117 belt set, the only datable example, which was manufactured in the first quarter of the fifth century.

In a textbook intended not for archaeologists, but for history students (p.xxiii), the author has a duty to make his readers aware that radical alternative interpretations of the evidence have been published. In this Myres fails. □

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CpG-rich islands and the function of DNA methylation

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It is likely that most vertebrate genes are associated with 'HTF islands'—DNA sequences in which CpG is abundant and non-methylated. Highly tissue-specific genes, though, usually lack islands. The contrast between islands and the remainder of the genome may identify sequences that are to be constantly available in the nucleus. DNA methylation appears to be involved in this function, rather than with activation of tissue specific genes.

A DINUCLEOTIDE sequence, it might be thought, is too short to be biologically interesting. The doublet CpG is exceptional, however, because of several features that are particularly evident in the DNA of vertebrates. One feature is rarity; CpG in bulk vertebrate DNA occurs at about one-fifth of the expected frequency^{1,2}. Rarity is almost certainly a consequence of the second feature: methylation. Between 60% and 90% of CpGs are methylated at the 5 position on the cytosine ring, and this accounts for most, if not all, of the methylcytosine in the vertebrate genome. Rarity of CpG is most probably caused by the failure of DNA repair mechanisms to recognize deamination of 5-methylcytosine to give thymine^{3,4}. This article concerns a third feature of CpG that has recently been uncovered: clustering of non-methylated CpG in G+C-rich 'islands'. A consideration of the properties of the clusters and their relationship to genes suggests that they may provide the key to understanding the function of DNA methylation.

Methylation-free islands

Evidence for clusters of non-methylated CpG has come from several directions. In one set of experiments it was shown that total DNA from a range of vertebrates contains a fraction of about 1% (the HTF fraction) that is extremely rich in cleavable sites for mCpG-sensitive restriction enzymes⁵. Sequences chosen at random from the HTF fraction of mouse DNA (but excluding ribosomal DNA) belong to islands of DNA several hundred base pairs long that contain CpG at more than ten times its density in bulk DNA⁶. The enrichment for CpG is due to a lack of CpG suppression in the HTF islands, and their relative G+C richness (65% G+C compared with 40% for bulk DNA). Three randomly chosen islands were characterized in this study, and all were found to be non-methylated in several mouse tissues, including sperm. Sequences flanking the islands were highly methylated. Absence of CpG suppression, of course, fits well with the lack of CpG methylation at HTF islands in germ cells. In spite of their common features, different islands do not appear to contain related nucleotide sequences. Instead, the majority of island sequences are 'unique' by the criterion of DNA reassociation⁶.

This kind of 'top-down' analysis of the whole genome has been paralleled by independent 'bottom-up' studies of individual genes. Early examples of genes whose 5' ends were found to lack methylation in all tissues are chicken $\alpha 2(1)$ collagen⁷, Chinese hamster adenosine phosphoribosyl transferase (APRT), and mouse dihydrofolate reductase⁸. In each of these cases sequence data have shown that CpG is concentrated in the non-methylated regions. Clusters of CpG are sufficiently distinctive to be identified by computer analysis, as in the case of human and mouse major histocompatibility antigen genes⁹. Methylation was not analysed in this study, but the discrete nature of CpG-rich regions was noted and a link with non-methylated DNA was proposed.

Incidence of islands

By the criterion of CpG frequency, there are now many genes with HTF-like sequences at the region where transcription originates (see Table 1; Fig. 1). A test of HTF character is to determine the G+C content of a likely region of several hundred base pairs, and then to count CpGs and, as a control, GpCs. If the G+C content is over 50%, and if CpG roughly equals GpC (both being close to the expected level in a sequence of this base composition), then the region is HTF-like. Note that this assay ignores the second criterion for an HTF island, lack of methylation, and in many of the examples cited methylation has not yet been investigated (see Table 1). The firm prediction is that HTF-like regions will prove to be non-methylated in germ cells at least. The list of genes which score positively in the test is dominated by genes that qualify as 'housekeeping', but it includes several genes that are not accurately described by this epithet. Many of those genes which are expressed exclusively in one or a few tissues (for example, β -globin, α -amylase, δ -crystallin and vitellogenin) are not represented (see Table 2).

If the present trend continues, most mammalian genes will turn out to be associated with HTF islands. Equating the approximate number of islands (about 30,000⁶) with the anticipated number of genes (20,000–50,000) in turn suggests that a high proportion of all islands will be gene-associated. In visualizing the relationship between HTF islands and genes it is important to realise that islands are large (between 500 and at least 2,000 base pairs) and so cover an extended region of the 5' end, often including the first few exons as well as upstream sequences (Fig. 1). Not all islands, however, surround the 5' end of the coding region. The glucose-6-phosphate dehydrogenase (G6PD) gene has a pair of islands close to the 3' end of the gene^{10,11}. There appears to be no consistent relationship between genes with CpG-rich 5' regions and the presence of TATA boxes; some genes on the list have obvious TATA boxes, others do not.

Methylation and transcription

There is evidence that transcription of genes with HTF islands is inhibited when the island is methylated. Transfection experiments with an artificially methylated hamster APRT gene demonstrate that methylation of the body of the gene does not interfere with transcription, but methylation of the 5' region, which is an HTF island, markedly reduces the level of transcription¹². Further evidence for the inhibitory effect of methylation at islands is provided by studies of genes on the inactive X chromosome. On this chromosome alone, HTF-like sequences have been found in the methylated state for both HPRT and G6PD genes^{11,13,14}. Methylation of non-island regions shows no obvious correlation with inactivity. Transfection experiments which tested the ability of purified DNA to confer the HPRT⁺ phenotype on HPRT⁻ recipient cells showed that the HPRT gene on the somatic inactive X is unable to express. On the

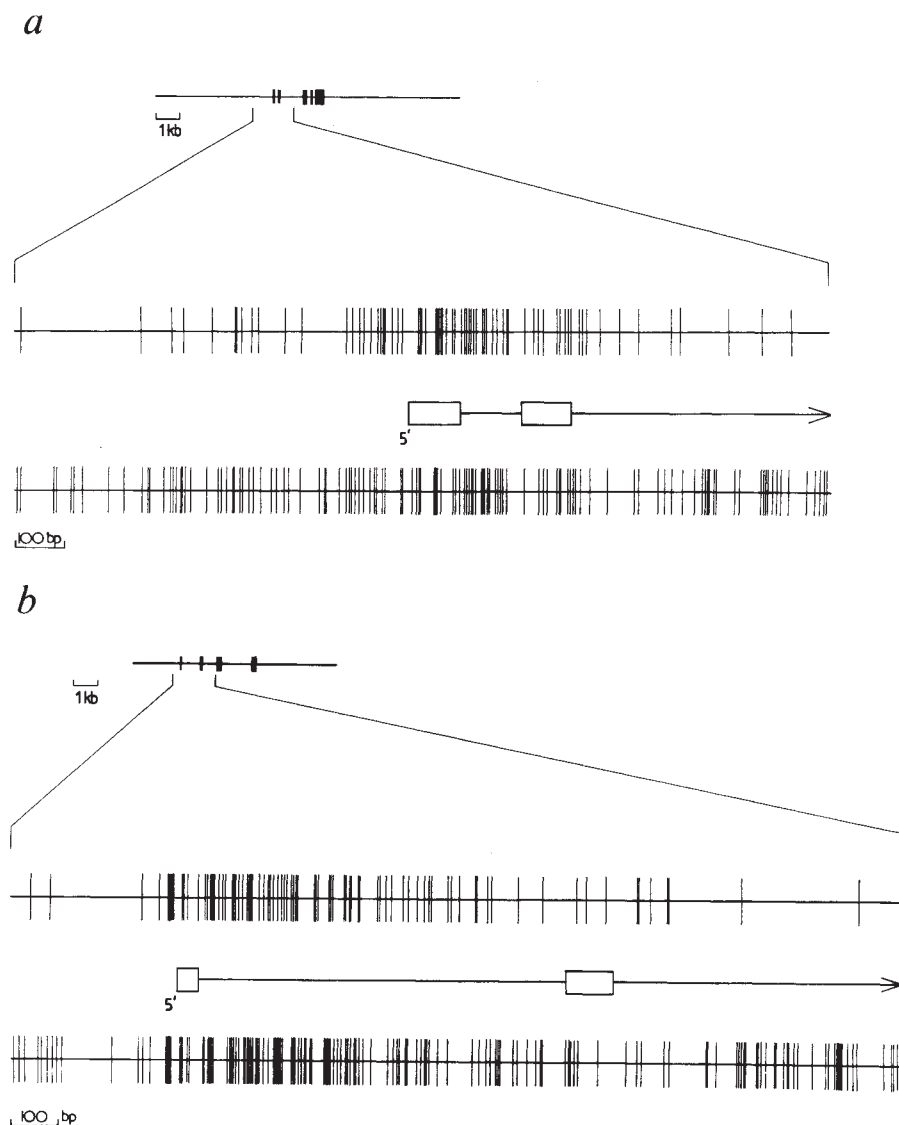


Fig. 1 CpG-rich islands at two mouse genes. *a*, Mouse adenine phosphoribosyl-transferase gene⁵⁹. Methylation studies of this gene have not been reported, but the Chinese hamster gene is known to be CpG-rich and unmethylated at the region corresponding to the mouse CpG-rich region⁸. *b*, Mouse ribosomal protein gene L-32⁶⁰. Limited methylation studies did not test whether the CpG-rich region is unmethylated in sperm. Each vertical line on the expanded scale represents a CpG doublet (upper map) or a GpC doublet (lower map). CpG is 4–5 fold deficient except in the island region where the frequency increases about tenfold. The GpC maps are shown for comparison to emphasize the relative deficiency of CpG outside islands. They show also that CpG and GpC frequencies are roughly equal in the island regions.

other hand, DNA from active X chromosomes or from sperm can transform cells to HPRT⁺^{15–17}, as can DNA from an inactive X chromosome that has been re-activated by the inhibitor of DNA methylation 5-azacytidine¹⁸. Methylation studies on the re-activated genes show that the islands are now unmethylated¹⁰. This evidence strongly suggests that methylation of the HTF island is a key part of suppressing the HPRT gene on the inactive X chromosome, although it is not clear that methylation is involved in events which initiate the inactivation process (see ref. 19). The important point for this discussion is that methylation of HTF islands apparently inhibits expression of the associated gene.

Viruses are relevant to a discussion of HTF islands because many viral sequences are HTF-like. Retroviral long terminal repeats, adenovirus and Herpes viruses are G+C rich and have CpG at close to the expected frequency. Moreover, both adenovirus and Moloney retrovirus are inactivated by DNA methylation^{20,21}. The absence of CpG suppression may simply mean that these viral genomes have evolved as non-methylated sequences. It is also possible, however, that the resemblance between certain viruses and housekeeping promoters is part of the viral strategy for propagation. Interestingly, simian virus 40 (SV40) and polyoma virus are low in G+C and are CpG deficient (resembling bulk vertebrate DNA), and both remain infectious when all CpGs are methylated²². The central role of T antigen in their life cycles may help to explain why these viruses do not mimic housekeeping-type promoters.

De novo methylation

Paradoxically, HTF islands contain methylatable cytosines (that is CpGs) at more than 10 times the frequency of bulk DNA, yet they must apparently remain free of methylation in order to permit expression of their associated genes. That islands are not intrinsically unmethylatable is demonstrated by their methylation on the inactive X chromosome. What, then, determines whether an HTF island is methylated or unmethylated? Little is known about the molecular events leading to *de novo* methylation of DNA, but the data imply that most cell types are capable of methylating accessible CpGs. Early embryonic cells and teratocarcinoma cells in culture are particularly active in this respect²³. This may be because *de novo* methylating activity is higher than usual at early embryonic stages, or alternatively *de novo* methylation may be a secondary consequence of the inactivity of certain genes in undifferentiated cells of this kind. The second explanation is favoured by the case of Moloney leukaemia provirus which is inactive in embryonic cells well before *de novo* methylation occurs^{24,25}. It seems that inactivity can invite *de novo* methylation. A simple possible explanation for this is that there are bound factors at HTF islands which normally exclude methylase and that inactivation of a gene can lead to displacement of the factors from the associated island, thus allowing access by the methylase.

The idea that *de novo* methylation is a secondary consequence of inactivation of an HTF-associated gene is attractive, for the

Table 1 Genes associated with HTF-like sequences

	Ref.	Species	Confirmed by methylation studies*
Genes for metabolic enzymes			
Hypoxanthine phosphoribosyl transferase		Mouse, human	(13, 14)
Adenine phosphoribosyl transferase		Mouse, hamster	(8)
Dihydrofolate reductase		Mouse, human	(8)
Glucose-6-phosphate dehydrogenase		Human	(10, 11)
HMG CoA reductase	(49)	Human	
Glyceraldehyde-3-phosphate dehydrogenase	(50)	Chicken	
Triose phosphate isomerase	(51)	Human	
Adenosine deaminase	(52)	Human	
Phosphoglycerate kinase	(53)	Human	
Cellular 'self-help' genes			
Metallothionien I		Mouse	
Superoxide dismutase	(54)	Human	
Heat shock gene	(55)	Human	
Cellular proto-oncogenes			
<i>c-int 1</i>	(56)	Human	A.P.B. and M. Taggart, unpublished
<i>c-myc</i>		Human, mouse	
<i>c-src</i>		Chicken	
<i>c-fos</i>		Mouse	
<i>c-Ha-ras</i>		Human	
Cellular structural protein genes			
Ribosomal protein L-30		Mouse	
Ribosomal protein L-32		Mouse	
β -Actin		Rat	
β -Tubulin		Rat, human	
Class I histocompatibility genes		Man, mouse	
Genes for tissue specific proteins†			
α 2(I) collagen		Chicken	
Retinol binding protein	(57)	Human	
α -Globin		Human, goat	A.P.B. and M. Taggart, unpublished
Thy-1	(58)	Human, mouse	F. Grosveld, personal communication
Class II histocompatibility genes		Human	

This partial list includes extensively sequenced genes which show HTF-like regions according to the criterion described in the text. See examples in Fig. 1. Un-referenced sequences are taken from the Genbank Sequence Database.

* In these cases *HpaII* and *HhaI* sites in the region were found to be cleavable in more than one tissue, including sperm. Methylation of some other genes on the list has been analysed, but these studies would not have tested CpG methylation in the CpG-rich region. Note that HPRT and G6PD are X-linked genes whose islands are methylated on the inactive X chromosome—see references. Also the 5' end of G6PD has not been identified, but there are HTF-like sequences near the 3' end.

† The protein products show tissue specificity, but for most genes it is not known whether transcription is also tissue specific. The *Thy-1* gene, for example, is transcribed at a low level in a number of mouse tissues (F. Grosveld, personal communication).

heritability of methylation patterns²⁶ will insure that inactivity persists in descendant cells even though the conditions that led to primary inactivation no longer exist. In other words, methylation could serve to imprint inactivity, allowing the silent state of the gene, once established, to be transmitted from one somatic cell generation to another or between generations of the organism (for example, inactive retroviral proviruses²⁷). Germline inactivation would also have longer-term consequences, as methylated sequences are hypermutable due to the evolutionary instability of mCpG. Over generations, CpGs would gradually be lost, and with them the HTF character of the sequence. Thus in the germ line, temporary inactivation of an HTF island could lead to prolonged inactivation by methylation and this in turn would accelerate the mutation rate at the island leading perhaps to irretrievable loss of the potential for activity.

Inactivation of this kind may reflect the original function of DNA methylation. The evidence from non-vertebrates, though sparse, does not conflict with this idea. The methylated fraction in non-vertebrate genomes has not yet been found to contain transcriptionally active sequences, as the invertebrate genes that have been examined so far all belong to the non-methylated fraction of the genome²⁸. The possibility exists, therefore, that methylation is a silencing mechanism in invertebrates, and that inactivation of viral genomes in vertebrates reflects persistence of this ancestral function.

Function and evolution

How did HTF islands arise? It is likely that their evolution is causally related to the increased methylation of DNA in vertebrates compared with the DNA of non-vertebrate ancestors²⁹. A plausible evolutionary scenario is that the spread of methylation through the genome led to depression of the general level

Table 2 Genes showing no evidence of HTF-like sequences

	Species
β -Globin gene family	Human, mouse, rabbit, goat
Placental lactogen	Human
Nerve growth factor	Human
α -amylase I and II	Mouse
Growth hormone	Mouse
Insulin	Mouse
Leukocyte interferon (α -a)	Mouse
Fibrinogen	Rat
δ -Crystallin	Mouse
Myoglobin	Seal

This partial list includes extensively sequenced genes (see Genbank Database) which are CpG-deficient throughout the gene and its 5' and 3' flanks.

of CpG by mutation. Many regions, for example promoters of housekeeping genes, stayed free of the repressive effects of methylation, perhaps with the aid of steric hindrance by permanently bound factors. Consequently, CpG remained frequent in these regions; an effect which was accentuated by a shift in the base composition of islands in favour of G+C.

The scenario does not require that the islands have a positive function in the cell. They could merely reflect the evolutionary footprint of DNA-binding proteins of various kinds that have consistently excluded the methylase. Alternatively, it is possible that the evolution of vertebrates has seen the essentially negative function of DNA methylation acquire an added dimension, by turning lack of methylation into a positive 'virtue'. To be more specific, the high density of unmethylated CpG which islands have in common could be the basis of a mechanism that renders

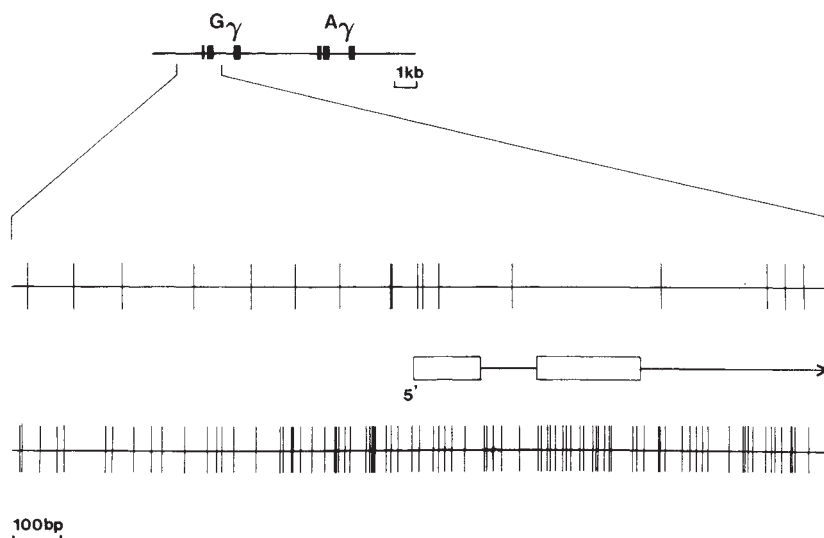


Fig. 2 Absence of a CpG-rich island at a human gene (the γ -G globin gene) that is tissue-specifically expressed. Sequence data is from Shen⁶¹. Comparing CpG and GpC maps (upper and lower, respectively) shows that CpG is deficient throughout this part of the gene. Other regions of the β -globin locus do not show evidence of CpG clustering either. Not all tissue specific genes lack CpG clusters. The human α -globin genes, for example, each have pronounced clusters of CpG (see Table 1).

all CpG-rich sequences constitutively 'available' for interaction with other cellular components. Despite our ignorance of the meaning of 'available' at the molecular level, we can imagine that CpG-rich DNA might assert itself in chromatin via CpG-binding proteins (see below), or because CpG-rich DNA has intrinsic properties which affect chromatin structure directly. Whichever mechanism is involved in mediating their function, the data require that the effect of islands on availability should be reversed by methylation.

The characteristics of HTF islands can be brought together in a speculative model. The model supposes that the availability of island sequences is achieved by bound factors (proteins) and that these same factors sterically exclude the methylase. The factors are presumed to have an affinity for sequences containing CpG, and to lose this affinity if CpGs are methylated. This means that for an inactive island methylation excludes the factors, while for an active island the factors exclude methylation. A system of this type would ensure that both the active and the inactive forms of an island are intrinsically stable states that would interconvert only with difficulty. The large size of islands compared to proteins suggests that multiple factors are bound, and these may interact co-operatively with one another. A requirement for co-operativity would mean that most non-island DNA, with its widely dispersed CpGs, could not function as an island even when devoid of methylation.

Tissue-specific genes

The above analysis has little to say about tissue specific genes like γ -globin which have no obvious HTF character (Fig. 2). Genes of this type are usually part of the methylated silent majority of the genome. When activated, they appear to be bound to a complex of tissue specific factors which presumably accomplish what HTF islands can achieve using ubiquitous, non-tissue specific factors. Given that there are usually rather few CpGs near tissue specific genes, and since what CpGs there are are methylated in the germ line and therefore hypermutable (see, for example, ref. 30), one would not expect to find CpG or methylation built into the activation mechanism of genes of this type. This conclusion is heretical given recent interest in DNA methylation as a mechanism of controlling tissue specific genes, but as outlined below it is compatible with the available evidence.

The finding that many tissue-specific genes are less methylated in cells where they are expressed than elsewhere (reviewed in ref. 31) can be explained in two ways: either demethylation precedes transcription, or it follows transcription as a consequence. Attempts to distinguish between cause and effect by studying the time course of transcriptional activation have

favoured the view that methylation-loss at a limited number of testable CpGs follows transcriptional activation as an effect³²⁻³⁶. Contrary arguments, that loss of methylation is in fact a causal element in gene activation, arise from two kinds of experimental results: the inactivity of artificially methylated globin DNA in transformation experiments; the activation of genes by azacytidine.

The experiments of Busslinger *et al.*³⁷ make clear that methylation can inhibit expression of a human γ -globin gene in mouse L-cells. However, it is known from work on chicken globin genes that the 5' end of the globin gene in erythroid cells is complexed with several erythroid-specific factors³⁸, and these are presumably absent in the non-erythroid cells that were used (for technical reasons) to test the effects of methylation on human gamma globin gene expression. (The endogenous globin gene in L-cells is not active). It follows that the expression of the introduced globin gene is probably mediated by factors that are usually involved in housekeeping gene expression. Since these factors normally operate at HTF islands, they may well be sensitive to CpG methylation, and this could explain the results. The experiment leaves untested the effect of methylation upon globin expression as it occurs in erythroid cells.

Apart from the globin experiments, the most suggestive evidence that loss of methylation may activate tissue-specific genes comes from the effects of azacytidine on cells (see ref. 39). Induction of differentiation in cells that normally show no tendency to differentiate is dramatic⁴⁰, but as yet unexplained at the molecular level. If demethylation of DNA is a primary cause of the observed effects (see ref. 39 for caveats), we need to know which gene is affected, and whether an *in vivo* process is mimicked.

At the single-gene level, few genes that qualify as tissue specific have been observed to react under the influence of azacytidine. Metallothionein I is an inducible protein involved in detoxification, and it can be activated by azacytidine in cell lines where the gene has become non-inducible⁴¹. However, this gene has properties more akin to a housekeeping gene than to a highly tissue specific gene, as most cell types express the gene at a low level⁴². Moreover the 500 base pair region surrounding the 5' end is HTF-like (59% G+C, no CpG deficiency) suggesting that this region lacks methylation in most or all tissues. Thus the methylation that inactivates the metallothionein gene in the cell line may not occur in the animal. Activation of globin genes by azacytidine has been seen^{43,44}, but the effect occurs in cells that are already predisposed to differentiate into red cells, and it can also be induced by agents that are not known to effect methylation. In non-erythroid cells, demethylation of globin genes by azacytidine does not lead to activation of the gene⁴⁵,

in agreement with earlier observations that globin genes are unmethylated in some cell types (placenta and HeLa cells) that are not noted for globin gene expression⁴⁶.

This critical survey of the evidence does not provide support for the prevailing view that specific loss of methyl groups is part of the process by which genes are developmentally activated. Most results would be accounted for if DNA-protein interactions like those which accompany transcriptional activity can interfere with the methylation process, leading to hypo-methylation of the active region. Passive demethylation of this kind, in regions where CpG is not clustered, might conceivably help to insure continued expression of an activated gene^{47,48}, or it might be of no consequence.

Conclusion

I have argued that HTF islands function to distinguish regions of the genome that are available for interaction with nuclear components in all cells from the unavailable remainder of the genome. The basis for the distinction is presumed to be CpG,

which is abundant and non-methylated in HTF islands, but infrequent and mostly methylated elsewhere. The evidence suggests that HTF-associated genes are inactivated if the islands become methylated. Methylation may be a secondary event following primary inactivation by other mechanisms, and may serve to imprint inactivity. For many genes that have been inactivated in this way, methylation appears to be the only impediment to transcription, since demethylation by artificial means is sufficient for re-activation. Some viruses may take advantage of cellular factors by mimicking the sequence properties of islands. This strategy, however, lays them open to inactivation by methylation. Genes that are expressed in a highly tissue-specific manner are not usually associated with HTF-like sequences. Demethylation is not sufficient to activate these genes, and may not be necessary either.

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ARTICLES

Changing the identity of a transfer RNA

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A leucine transfer RNA has been transformed into a serine transfer RNA by changing 12 nucleotides. This result indicates that a limited set of residues determine tRNA identity.

THE rules for specificity in protein synthesis are to be found in the specific interactions between transfer RNA molecules and aminoacyl tRNA synthetases (AAS)¹. There exists a single AAS for each of the 20 amino acids, but because of the degeneracy of the genetic code and the limited range of allowed codon-anticodon interactions², there are generally multiple tRNAs

specifying each amino acid. The accurate transfer of information from messenger RNA to protein therefore relies on each AAS recognizing and acylating its cognate set of tRNAs while failing to acylate all others. All tRNAs so far sequenced (and the number is greater than 300)³, can be folded into the familiar cloverleaf structure¹ and share a set of absolutely conserved

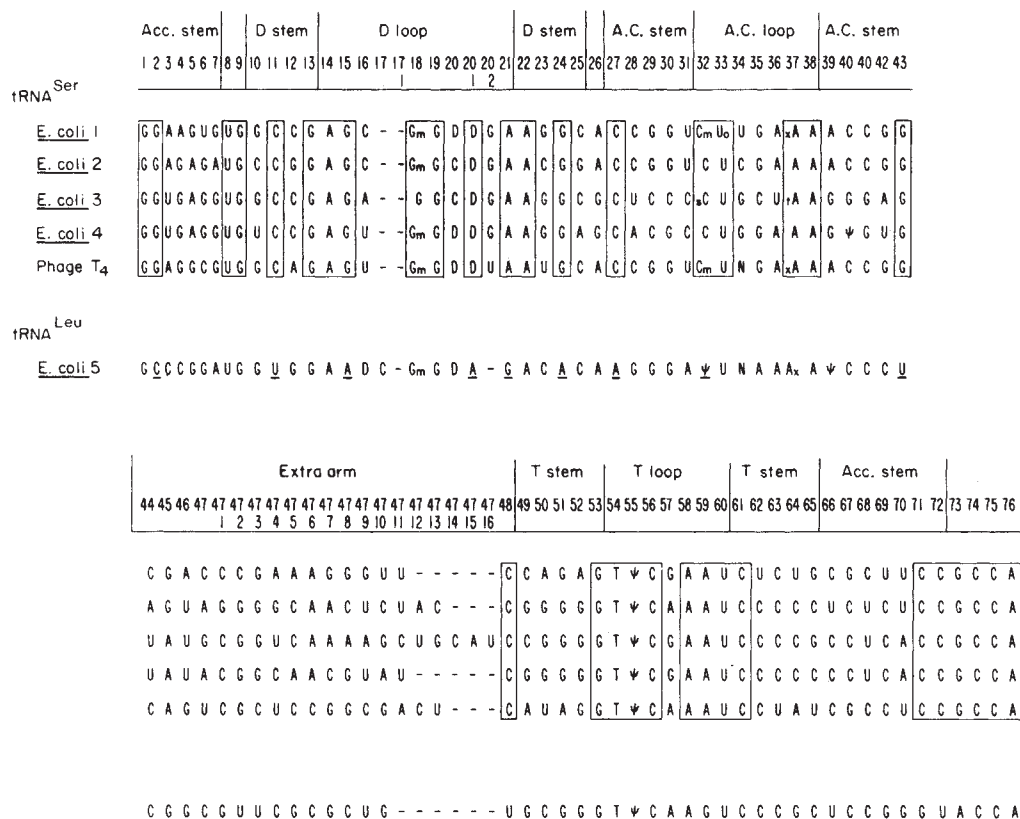


Fig. 1 Serine tRNAs recognized by *E. coli* serine AAS²⁹⁻³⁴ compared with *E. coli* tRNA^{Leu} (ref. 59 and K. Jones and B. Reid, personal communication). Boxed nucleotides indicate absolute conservation among the five tRNA^{Ser} sequences. The conserved serine nucleotides that differ from tRNA^{Leu} have been underlined in the tRNA^{Leu} sequence. A fifth *E. coli* serine tRNA has been sequenced (not shown) and is identical to that of *E. coli* 4, except for residue 20, which is a C³⁹. Acc. stem, acceptor stem. A.C., anticodon.

nucleotides. The X-ray crystal structures of several tRNAs⁵⁻⁸ show that these constant nucleotides are involved in stabilizing the tertiary structure of the tRNA molecule, therefore, by extrapolation, the tertiary structures of all of the tRNAs in the cell are very similar. In some way, within the variations allowed by this framework, there exists a set of distinguishing features which constitute amino-acid specificity, that is, the identity of each tRNA.

By contrast, aminoacyl tRNA synthetases are quite variable in structure; they range in size from 300 to 1,000 amino-acid residues and are found in various quaternary forms⁹. Despite the variability of these enzymes, a unifying model has been proposed for the interaction between the tRNA molecule and AAS. Summarizing diverse chemical and physical studies involving several cognate complexes¹⁰ (see also refs 9, 11, 12), Rich and Schimmel proposed that all AAS interact with tRNA along the inside diagonal of the L structure^{5,6}, the regions of contact inferred from these studies extend from the acceptor stem through the D stem to the anticodon loop. While the range of AAS contacts may be similar for all tRNA substrates, the rules for recognition lie in the detailed interactions and these must, of course, be idiosyncratic. For example, while the anticodon is clearly an important recognition element for the *Escherichia coli* tRNA^{Met} synthetase¹³⁻¹⁵, this seems less likely to be the case of the serine and leucine synthetases because there are six codons specifying each of their respective amino acids, requiring a set of isoacceptor tRNAs with different anticodons.

A genetic approach to understanding AAS-tRNA recognition was taken over 15 years ago¹⁶⁻¹⁹. In those experiments, a genetic selection was devised to isolate mutant tRNAs having altered specificity. In the initial experiments, the *E. coli* amber suppressor gene *supF* was used. This suppressor is a mutant allele of one of three tRNA^{Tyr} genes in *E. coli* and can recognize the amber codon UAG because it has an altered anticodon, CUA²⁰ (for a review of nonsense suppression, see ref. 21). Among the amber mutants in bacterial and phage genes, there are some which can be suppressed by *supD* (serine) and *supE* (glutamine) but not by *supF*. Selection of *supF* mutants which could suppress

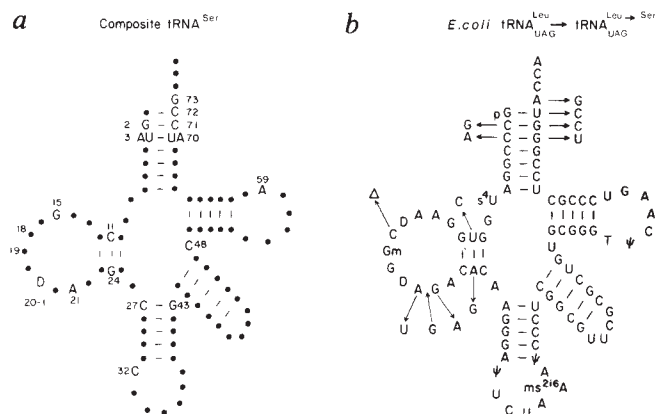
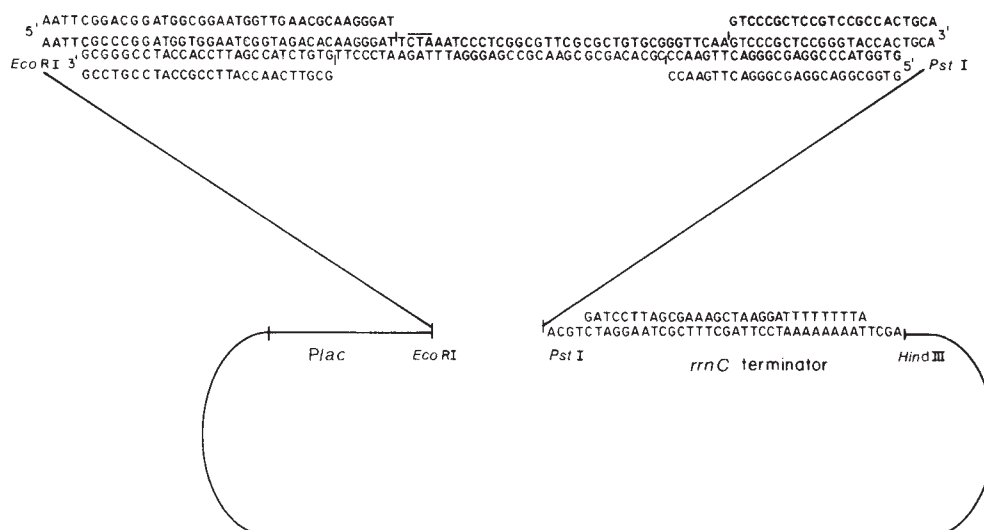


Fig. 2 a, Cloverleaf representation of conserved *E. coli* tRNA^{Ser} residues. The indicated nucleotides are those that are absolutely conserved in all six serine tRNAs and not found in tRNA^{Leu}. b, tRNA^{Leu} with the changes proposed to confer tRNA^{Ser} specificity. The modified nucleotides are those present in tRNA^{Ser}. Note that the changes in the D loop at positions 20-2 and 21 are equally well represented by insertion of an A between residues 21 and 22. Thus, there are actually 11 independent changes.

this class of ambers resulted in tRNA^{Tyr} alleles with altered specificity. The mutations occurred in the first and second base pairs of the acceptor stem and in the fourth nucleotide from the 3' end of the tRNA^{16,17,22-25}. In all cases, the amino acid inserted by the mutant tRNAs was glutamine. This interesting result was somewhat obscured later by the finding that amber suppressor alleles of the tRNA^{Trp} gene also insert glutamine²⁶. One interpretation of these results is that the glutamine synthetase is indiscriminate and charges substrates which are not recognized by other synthetases. Other alterations in tRNA^{Tyr} specificity were never observed, perhaps because too many changes are required. The approach remains an attractive one, however, because the alteration of identity with retention of

Fig. 3 The tRNA^{Leu}_{UAG} gene was constructed from six oligonucleotides containing the tRNA^{Leu}_{UAG} sequence flanked by *Eco*RI and *Pst*I restriction endonuclease cohesive ends at the immediate 5' and 3' ends of the gene respectively. Junctions between oligonucleotides are indicated. The four oligonucleotides containing the appropriate sequence alterations for construction of tRNA^{Leu→Ser}_{UAG} exactly replace four of the tRNA^{Leu}_{UAG} oligonucleotides, and are shown above and below the tRNA^{Leu}_{UAG} sequence.

Methods. Deoxyoligonucleotides were synthesized on an Applied Biosystems 380A DNA synthesizer. Three to five A₂₆₀ units of oligonucleotide were electrophoresed through a 20% acrylamide/7 M urea gel, visualized by ultraviolet shadowing, excised and eluted in 0.3 M NaCl, 10 mM Tris-HCl pH 7.4, 1 mM EDTA, 1% phenol at 37 °C overnight. The DNA was precipitated with 100% ethanol, washed with 70% ethanol, dried and resuspended in dH₂O. Phosphorylations were carried out on 2 µg of each oligonucleotide in 70 mM Tris-HCl pH 7.6, 10 mM MgCl₂, 5 mM dithiothreitol (DTT), 100 µM ATP, 100 µCi ³²P-ATP, with four units of polynucleotide kinase for 1 h at 37 °C, followed by phenol/chloroform extraction and ethanol precipitation. Eighty pmol of each phosphorylated oligonucleotide were incubated together in 100 mM NaCl at 80 °C for 5 min, then allowed to cool to room temperature over a period of 3 h. The mixture was made 50 mM in NaCl and combined with digested vector in a 10:1 insert to vector (mass) ratio. The vector in this case was M13mp8 containing a synthetic *rrnC* terminator⁴⁰ inserted into the *Pst*I–*Hind*III sites. Use of this transcription terminator was suggested by the work of Yarus *et al.*⁶⁰. The sequence of the terminator, synthesized as two chains, is indicated. Ligation was carried out in 50 mM Tris-HCl pH 7.6, 10 mM MgCl₂, 20 mM DTT, 1 mM ATP, 50 µg ml⁻¹ bovine serum albumin with 1 unit of T₄ DNA ligase at 15 °C for 12 h. The ligation mixture was used to transform competent JM101 (*F'* *traD36 proAB lacI^q Zm15/Δ(lac pro) supE thi*). Single-stranded DNA from the resulting plaques was immobilized on nitrocellulose filters⁶¹ and screened for the presence of inserts by first prehybridizing in 10× Denhardt's reagent⁶², 0.2% SDS, 6×SSC⁶³, 1 mM EDTA for 2 h at 65 °C, then hybridizing with 4×10⁵ Cerenkov c.p.m. ml⁻¹ of one of the internal oligonucleotides in 5×Denhardt's reagent, 6×SSC at 50 °C for 12 h. The filters were washed in 6×SSC at room temperature for 5 min, then at 50 °C for 5 min and autoradiographed. Single-stranded DNA was prepared from positive candidates and sequenced according to the method of Sanger *et al.*⁶⁴.



tRNA function, by definition, reveals elements that determine specificity.

The advances in oligonucleotide synthesis allowing rapid gene synthesis²⁷ have prompted a return to the genetic approach to studying AAS-tRNA interaction. If multiple changes are required to alter the specificity of a tRNA, they cannot be selected but they can be constructed. This is the approach we have taken here.

Rationale

To define the specific interactions between *E. coli* serine-specific tRNAs and their cognate AAS, we set out to transform a leucine-specific amber suppressor tRNA into an amber suppressor tRNA recognized exclusively by the serine AAS. We assume that there exists a limited set of changes one could make to a leucine tRNA that would entirely block its recognition by the leucine AAS and allow recognition solely by the serine AAS. The choice of this particular transformation was in part dictated by the fact that serine and leucine amber suppressor alleles exist in *E. coli*, and because all serine and leucine tRNAs fall into the type II structure class. Type II tRNAs have large extra loops in a stem-loop configuration and therefore lack the triple-base tertiary interactions which are found in type I tRNAs²⁸. In addition, it is possible that the extra loop is a distinct structural feature recognized by the AAS for type II tRNAs, although this notion has not been addressed experimentally.

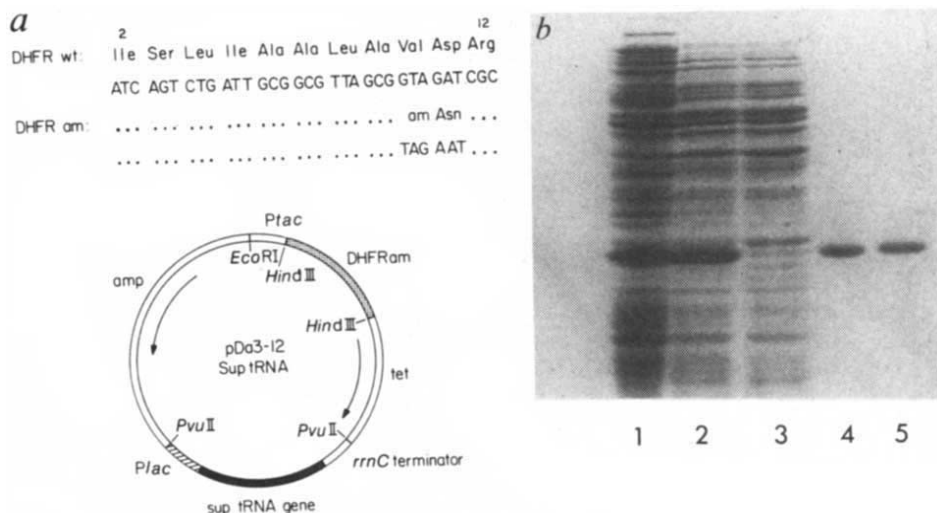
Six different tRNA^{Ser} isoacceptors recognized by the *E. coli* serine AAS have been sequenced (five *E. coli* tRNA^{Ser} species and a T4 tRNA^{Ser}; refs 29–34). Comparison of these sequences provides the information necessary to design the transformation. Such an approach was used previously to delineate sequences important in the recognition of tRNA^{Phe} by the yeast phenylalanine AAS^{9,35}. Inspection of the serine tRNA sequences reveals 35 positions in which a nucleotide is absolutely conserved (Fig. 1). It is likely that the specific residues that are necessary

and sufficient for recognition of the set of tRNA^{Ser} molecules by serine AAS are limited to these 35 nucleotides. Of these, 14 occur in all tRNAs (the constant nucleotides) and an additional seven are present in tRNA^{Leu}_{UAG}. The remaining 14, detailed in Fig. 2a, are concentrated in the acceptor stem and D arm, together with one each in the TψC loop, extra arm, and anticodon loop, and two comprising a base pair in the anticodon stem.

Figure 2b shows the proposed changes for the Leu→Ser transformation, the justifications for which are as follows. We chose to focus only on the acceptor stem and D stem and loop as these domains have been shown to be in contact with the synthetase in all cases studied. In this initial exercise, we have ignored the TψC stem-loop, the extra arm and the anticodon stem and loop. Although the anticodon has been strongly implicated in some tRNA-AAS interactions^{13–15,26,36,37}, the existence of at least six isoaccepting species of serine tRNA, recognizing six different codons, and the existence of a serine tRNA suppressor allele, argues against a crucial role for the anticodon region in the case of serine tRNA recognition. Accordingly, the conserved tRNA^{Ser}-specific bases at positions 32 in the anticodon loop and the base pair 27–43 in the anticodon stem were not incorporated into the tRNA^{Leu}_{UAG} framework. We have thus superimposed on tRNA^{Leu}_{UAG} the conserved serine nucleotides at positions 2, 71, 72 and 73 in the acceptor stem as well as positions 20–1, and 21 in the D loop, and base pair 11–24 in the D stem.

Looking at probable tertiary interactions in the leucine and serine tRNAs, there are five base-pair interactions common to both type I and II tRNAs which appear to be responsible for stabilization of the tertiary structure^{5,6,28}; four of these, G18·U55, G19·C56, U54·A58 and U8·A14, involve constant nucleotides, and are therefore not directly affected by the alterations we propose. The fifth, a transtertiary base pair between residues 15 and 48, is G15·C48 in all serine tRNAs. As this

Fig. 5 a. The 19-mer 5'-ACGTA-AAGCTTAACCGTC-3' was used to create a *Hind*III site in between the ribosome binding site and Pribnow box of the *E. coli fol* gene (C. R. Morris, unpublished results) encoded by a 1-kilobase fragment contained in M13mp8 (ref. 66). This was done to facilitate subcloning of the gene into a *tac* expression vector. Subsequently, the 23-mer 5'-GATAACGCGATTCTACGCTAACG-3' was used for oligonucleotide-directed mutagenesis of the *fol* gene. The 247-base pair (bp) *Eco*RI-*Hind*III fragment of *ptac*12H⁵³ containing the *tac* promoter was ligated into the *Eco*RI-*Hind*III site of pBR322 to create pTAC10. Subsequently, the DHFR amber, encoded by a 1,057-bp *Hind*III fragment, was subcloned into the *Hind*III site of pTAC10, yielding pDA3-12. The 392-bp *Pvu*II fragment from pBR322- β am:tRNA^{Leu→Ser}_{UAG} or pBR322- β am:tRNA^{Leu→Ser}_{UAG} containing the *lac* promoter, synthetic tRNA gene and *rrnC*-derived terminator, was subcloned into the *Pvu*II site of pDA3-12 to yield pDA3-12:Leu or pDA3-12:Leu→Ser. **b.** DHFR purification visualized by Coomassie blue staining of an SDS-polyacrylamide gel. Two litres of MC1061 (*lac*_{x74} *galU galK rpl Δ(araABIOc-leu₇₆₇₉) hsr⁻ hsm⁺*) carrying pTAC101 (the same construct as pDA3-12, except that the *fol* gene is wild type) in L broth were incubated at 37 °C with aeration to an *A*₆₀₀ of 0.4–0.6 isopropyl thiogalactoside was added to 1 mM and the culture was incubated as before for an additional 4 h. Cells were harvested and lysed⁶⁷ (lane 1) and the crude lysate was extracted with ammonium sulphate⁶⁸. The 90% ammonium sulphate-saturated insoluble fraction (lane 2) was brought to a protein concentration of 20 mg ml⁻¹ and dialysed with 3.2 ml of methotrexate-Sepharose⁶⁹ overnight. The resin was batch-washed (lane 3 is the initial dialysate) and DHFR eluted with folic acid (lane 4)⁶⁹. To remove nucleic acid and folate, peak fractions were dialysed against 50 mM potassium phosphate pH 8.0, 1 mM DTT and applied to a DEAE-Sepharose column (1 ml per mg protein) equilibrated in the same buffer. DHFR was eluted in a linear gradient of 0–0.4 M KCl (lane 5), dialysed against water and lyophilized. Mutant DHFR was purified in a similar manner but on a larger scale (12 l for the tRNA^{Leu→Ser}_{UAG} substituted mutant) and the peak fractions from the DEAE column were passed through methotrexate affinity and DEAE columns a second time.



had been wrong by one or two nucleotides, the serine-specific amber could have been used to select an active serine suppressor by spontaneous or induced mutation. Alternatively, this mutant could be used to select stronger variants of a weak serine suppressor. In the second approach the tRNA^{Leu→Ser}_{UAG} suppressor was used to suppress an amber mutation near the beginning of the *E. coli* dihydrofolate reductase gene *fol*, and subsequently the suppressed protein was sequenced.

Serine-requiring amber mutation

The active site of β -lactamase contains an essential serine residue^{45,46} which participates in the cleavage of the β -lactam ring of ampicillin and thereby confers antibiotic resistance to cells harbouring the β -lactamase gene *amp*. By oligonucleotide mutagenesis we converted the AGC serine codon at residue 68 (residue 70 according to Ambler's nomenclature⁴⁷) of the pBR322 *amp* gene to TAG (Fig. 4a). Figure 4b shows that this mutation can be suppressed in an *E. coli supD* strain (*supD* inserts serine) but not by *supE* (glutamine), *supF* (tyrosine) or *supP* (leucine). The only other amino acids which might be expected to substitute for serine at this position, based on some knowledge of the reaction mechanism⁴⁸, are cysteine and threonine. These substitutions have been made^{46,49–51} and in the case of cysteine resulted in an enzyme with 1/30 of wild-type activity. In the case of threonine, the enzyme was inactive⁵¹. The active-site amber mutation therefore meets our requirements as apparently it can be functionally suppressed only by a serine-inserting suppressor.

The synthetic suppressor genes tRNA^{Leu→Ser}_{UAG} or tRNA^{Leu→Ser}_{UAG}, together with the *lac* promoter and the *rrnC* terminator, were ligated into the *Pvu*II site of pBR322- β am. Each plasmid was introduced into *E. coli* and the transformants were tested for growth on tetracycline and ampicillin. Only the tRNA^{Leu→Ser}_{UAG} suppressor allowed growth on ampicillin (Fig. 4c). Furthermore, the cells containing tRNA^{Leu→Ser}_{UAG}-suppressed β -lactamase are fully viable in the presence of the drug at 80 μ g ml⁻¹. We conclude that tRNA^{Leu→Ser}_{UAG} is a functional tRNA which inserts serine. This experiment cannot, however, exclude the possibility

that the tRNA inserts other amino acids also. The only way to determine the specificity of suppression is to sequence through the site of the amber mutation in a suppressed protein.

tRNA^{Leu→Ser}_{UAG} specificity

To determine the specificity of the tRNA^{Leu→Ser}_{UAG} suppressor, we needed to use it for suppression of an amber mutation close to the beginning of a gene whose product is relatively small, easy to purify, and present at high concentration. The *E. coli* dihydrofolate reductase (DHFR) gene, *fol*, proved an attractive candidate. We linked the *fol* gene, the sequence of which is known⁵², to the *tac* promoter⁵³ in the expression vector pTAC10 (Fig. 5). The 159-amino-acid DHFR protein represents approximately 10% of the total protein in fully induced strains of *E. coli* harbouring this plasmid, and is easily purified by methotrexate affinity chromatography. An amber mutation at a site near the N-terminus would allow sequence determination of the suppressed DHFR by Edman degradation⁵⁴ of the intact protein. Residues 2–8 constitute a strand of a β -pleated sheet and are part of the NADP binding site. We chose position 10, a valine residue found in a loop on the surface of the protein, because this residue is not phylogenetically conserved and X-ray crystallographic data reveal that it is well removed from the active site^{55–57}. Substitution of any residue at this site should not affect the purification of DHFR on a methotrexate affinity column. Another point to consider is that the efficiency of amber suppression and therefore the yield of suppressed protein is dependent on the context of the amber codon. UAG codons followed by an A residue are usually better suppressed than those followed by a G⁴². Consequently, by oligonucleotide-directed mutagenesis, we altered the DHFR sequence GTA-GAT of codons 10 and 11 to TAG-AAT, changing the protein sequence from Val-Asp to amber-Asn. This altered *fol* gene, together with its *tac* promoter, was transferred to plasmids carrying either the tRNA^{Leu→Ser}_{UAG} or tRNA^{Leu→Ser}_{UAG} genes (Fig. 5a). Figure 5b documents the purification of DHFR from a strain harbouring the wild-type gene under control of the *tac* promoter (pTAC10).

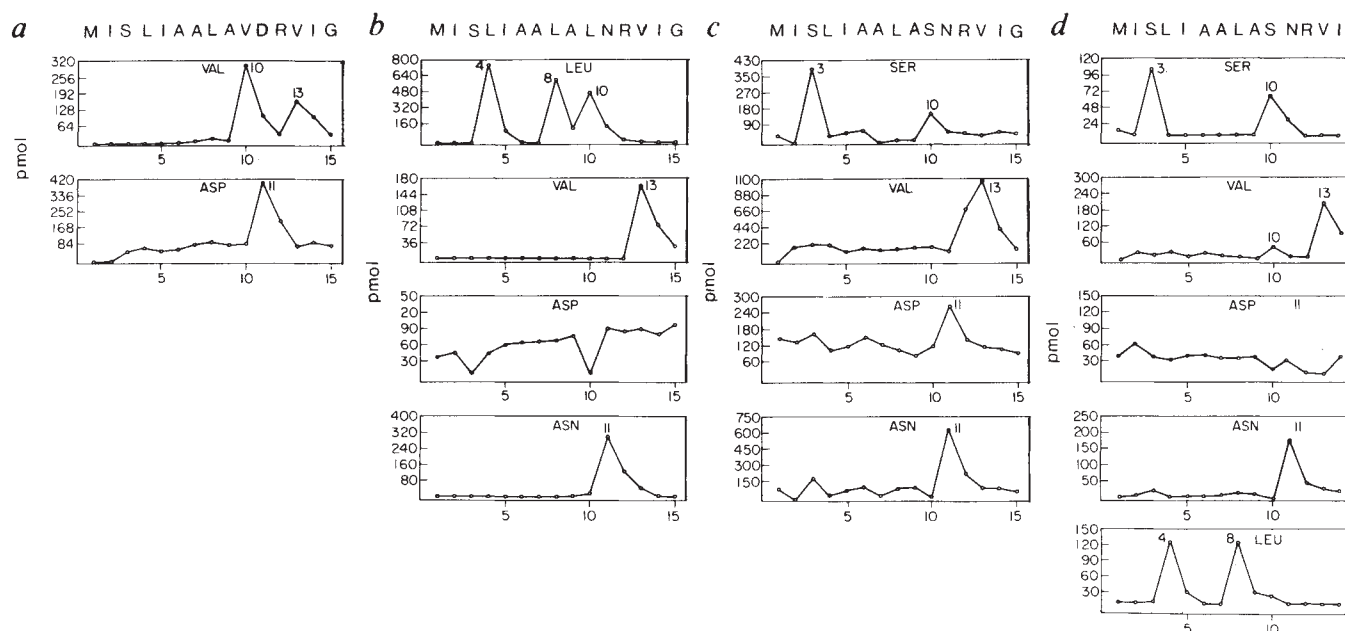


Fig. 6 Protein sequence analysis of wild-type DHFR (a), DHFRam/Leu suppressor (b), DHFRam/Ser suppressor (c) and DHFRam/Leu → Ser suppressor (d). Protein sequence analysis was carried out on an Applied Biosystems Model 470A gas-phase protein sequencer⁵⁸ by the USC Microchemical Core Laboratory. The picomolar yields of phenylthiohydantoin (PTH) derivatives of valine, aspartic acid, asparagine, leucine and serine are plotted against the residue number for wild-type DHFR, isolated from *E. coli* host carrying pTAC101, and mutant DHFR isolated from strains carrying pDA3-12:Leu (b) or pDA3-12:Leu → Ser (d), as well as from a *supD* strain carrying pDA3-12 (c). The single-letter amino acid code is used on the x axis.

Dihydrofolate reductase was purified from four strains: one carrying pTAC101, another a *supD* strain harbouring the DHFR amber mutant in the *tac* expression vector (pDA3-12), and the third and fourth carrying pDA3-12:Leu and pDA3-12:Leu → Ser, respectively. Each purified protein was sequenced by Edman degradation using an automatic gas-phase sequencer⁵⁸. In interpreting these results, it should be considered that the chromosomal copy of the *fol* gene is present in these strains, leading to fractional yield (usually small) of Val-Asp at positions 10 and 11. This sequence is very clearly seen when wild-type DHFR is sequenced but when the DHFR amber is suppressed by the tRNA^{Leu}_{UAG} suppressor, the sequence is unambiguously Leu-Asn at positions 10 and 11. Overproduction of the plasmid-encoded DHFR is probably greater than 100-fold and the tRNA^{Leu}_{UAG} suppressor is efficient. When the DHFR amber is suppressed by *supD*, serine is clearly present in position 10, demonstrating that the well known problems in detecting serine in Edman degradation have been overcome. Here, valine is clearly not detected at position 10 but there is some aspartate at position 11; we believe this results from deamidation of asparagine.

When the DHFR amber is suppressed by the tRNA^{Leu→Ser}_{UAG} suppressor, there is a good yield of serine at position 10, but also a detectable (12–15%) amount of valine. The presence of valine is due to the fact that the tRNA^{Leu→Ser}_{UAG} suppressor is weak, giving rise to a lower ratio of suppressed protein to wild-type protein. There is also a small amount (11%) of leucine at position 10; this is probably due to carry-over of the leucine from position 8, but the experimental method used cannot exclude the possibility that the tRNA^{Leu→Ser}_{UAG} suppressor inserts small amounts of leucine or valine. We could detect no other amino acid at position 10, and conclude that we have changed a strong leucine suppressor to a weak serine suppressor.

Discussion

Comparison of the sequences of six serine tRNAs which are substrates for the *E. coli* serine AAS reveals several conserved nucleotides. Here we altered an amber suppressor allele of

tRNA^{Leu}₅ at 12 positions to yield a tRNA sequence containing a subset of these conserved serine residues—those located in the acceptor stem and in the D stem and loop. The resultant molecule is a weak suppressor which inserts serine in response to amber nonsense codons. The specific acquisition of tRNA^{Ser} identity was established by a highly discriminatory functional assay, while the loss of tRNA^{Leu} identity in exchange for unique acquisition of tRNA^{Ser} identity was established by protein sequencing. Our study demonstrates that a limited number of nucleotides by virtue of their nature and position on a tRNA scaffold, are sufficient to dictate cognate synthetase recognition. It is noteworthy that this result was achieved with only primary and secondary structure information about the two tRNAs and no knowledge of the AAS structures.

A number of questions remain of course. We have converted a strong suppressor into a weak suppressor. This reduction in efficiency may be the result of a weak interaction between the suppressor tRNA and serine AAS. Alternatively, it may reflect a perturbation in the general tRNA tertiary structure resulting in poorer competition with the release factors. There are two possible routes to producing a better suppressor with serine specificity. First, we can further alter the sequence by including those changes in the anticodon stem and T ψ C loop that were conserved in all serine tRNAs, but which we chose to ignore. Our predictions about the importance of specific tertiary interactions in AAS recognition also warrant a more systematic investigation. Perhaps a better method, however, is to mutagenize the gene and select stronger serine suppressors by requiring suppression of the β -lactamase active-site amber in the presence of increasingly higher levels of ampicillin. When a strong serine suppressor is generated, the sequence of the tRNA will be determined. At that point, we can begin a systematic analysis of the identity switch to determine which changes were necessary. Eventually, the minimum set of changes required to transform tRNA^{Leu} to tRNA^{Ser} can be determined. This information will then be used to effect a different transformation, for example, tRNA^{Tyr} to tRNA^{Ser}. It will clearly be necessary to gather supporting *in vitro* kinetic and thermodynamic data for

the various tRNAs and AAS involved. This iterative method can lead to a more detailed understanding of the essence of serine tRNA identity.

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Correlations between T-cell specificity and the structure of the antigen receptor

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The derived amino-acid sequences of the heterodimeric antigen receptors expressed by a series of murine T-cell clones are presented. A comparison of the receptor sequences indicates that several mechanisms for generating receptor diversity can influence T-cell specificity, including junctional diversity, combinatorial joining, and combinatorial chain associations.

T LYMPHOCYTES recognize foreign antigens in association with cell surface molecules encoded by the major histocompatibility complex (MHC). This phenomenon, termed MHC restriction, distinguishes T-cell recognition from the direct antigen specificity of the immunoglobulin receptors on most B lymphocytes¹⁻⁴. Particularly useful in studying the molecular interactions involved in T-cell recognition have been T-cell immune responses in which the primary amino-acid sequences of both

the foreign antigen and the restricting MHC molecule have been determined. One well characterized system uses the antigen cytochrome c, a globular, haem-containing mitochondrial protein 103 or 104 amino acids long. The amino-acid sequences of cytochromes c from several species have been determined, and from insects to mammals there is little sequence diversity (Fig. 1a)^{5,6}.

The obligate recognition of antigen in association with MHC molecules is phenomenologically related to immune response polymorphisms that map to the MHC gene complex. The murine response to pigeon cytochrome c is under such immune response (Ir) gene control⁷⁻⁹; certain MHC congenic strains of mice

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Fig. 1 *a*, Amino-acid sequences of the carboxy-terminal CNBr fragments of the cytochromes *c* of mouse, pigeon, tobacco hornworm moth and tuna. Only those residues differing from the mouse sequence are shown. These fragments show immunogenic and antigenic properties similar to those of the entire molecules⁷⁻⁹. *b*, Differences in the predicted amino-acid sequences of I-E_β^k (ref. 56), I-E_β^s (ref. 15) and I-E_β^b (ref. 57). Only those residues differing from the sequence of I-E_β^k are given, with the amino-acid number noted above the sequence. Differences all occur in the membrane distal domain and are grouped, with the exception of amino acid 79, into allelic hypervariability regions (HVRs)⁵⁶. Residue 79 was originally placed outside any HVR. The available amino-acid sequence for I-E_β^s begins at residue 7; our analysis would ignore differences in the first six amino acids.

<i>a</i>	81	85	90	95	100
Mouse	Ile Phe Ala Gly Ile Lys Lys Gly Glu Arg Ala Asp Leu Ile Ala Tyr Leu Lys Lys Ala Thr Asn Glu				
Pigeon			Ala		Gln Ala Lys
Moth	Val	Leu	Ala Asn		Gln Lys ---
Tuna			Gly	Gln	Ser Ser ---

<i>b</i>	HVR I	HVR II	HVR III	HVR IV
	6 12 13	29	72 75 79	87 89 93
B10.A = I-E _β ^k	Pro Cys Lys	Val	Lys Glu Val	Phe Asn Pro
B10.S(9R) = I-E _β ^s	? Ser Thr	Glu	Arg Ala Tyr	Leu Lys
B10.A(5R) = I-E _β ^b	Leu	Glu		Ser Lys Arg

respond well while others respond poorly or not at all. There are two genes which influence this ability to elicit an immune response, one mapping to the *I-A* region of the murine MHC, the other to the *I-E* region^{7,10}. The glycoprotein products of these genes, I-E_β and I-E_α respectively, constitute the Ia molecule to which cytochrome *c*-specific helper T cells are restricted¹¹⁻¹⁴. Mapping studies have identified the recognition of the more polymorphic light chain, I-E_β, as being the critical factor in predicting the response to a given cytochrome *c* sequence. A comparison of the predicted amino-acid sequences encoded by three *I-E*_β alleles (Fig. 1*b*) suggests the importance of a hydrophobic fourth hypervariable region (HVR), particularly the amino acids at positions 87 and 93, in allowing a stable interaction between the I-E_β molecule and pigeon cytochrome *c*¹⁵. Conversely, using both chemically derived and phylogenetic variants of the carboxy-terminal 24 amino-acid peptide, the site on the cytochrome *c* molecule which may be involved in the interaction with I-E_β was mapped to residues 103 and 104 (refs 13, 16) and the site involved in the interaction with murine T cells mapped to residues 99 and 100 (refs 7, 8, 16, 17). Thus, in this *Ir* gene-controlled response to closely related peptide antigens, both the antigens and the Ia molecules have been completely sequenced, and a model has been proposed, based on functional data, for mapping the separate sites on the antigen which interact with the T-cell receptor (TCR) and the MHC molecule.

In contrast, little is known about the receptors used by cytochrome *c*-specific T cells. Monoclonal antibodies have shown that the TCR consists of two membrane-bound, disulphide-linked glycopeptides (termed α - and β - chains), each of relative molecular mass (M_r) ~40,000 (refs 18-22), noncovalently associated with several accessory molecules²³⁻²⁵. The genes encoding the α - and β -chains have recently been cloned; the β -chain gene consists of separate segments encoding the variable (V), diversity (D), joining (J) and constant (C) regions²⁶⁻²⁸. The α -chain gene is composed of V, J and C regions, although the existence of a D-region segment has yet to be excluded²⁹⁻³¹. As occurs in the maturation of immunoglobulin genes, the V, D and J segments somatically rearrange during differentiation to form the functional genes³².

We have cloned and sequenced complementary DNAs encoding the TCRs from a series of T cells having similar but distinct specificities for pigeon cytochrome *c* and I-E_β^k-encoded molecules. A comparison of the derived amino-acid sequences from these receptors provides the first information as to which regions of the TCR affect MHC restriction, antigen recognition and alloreactivity.

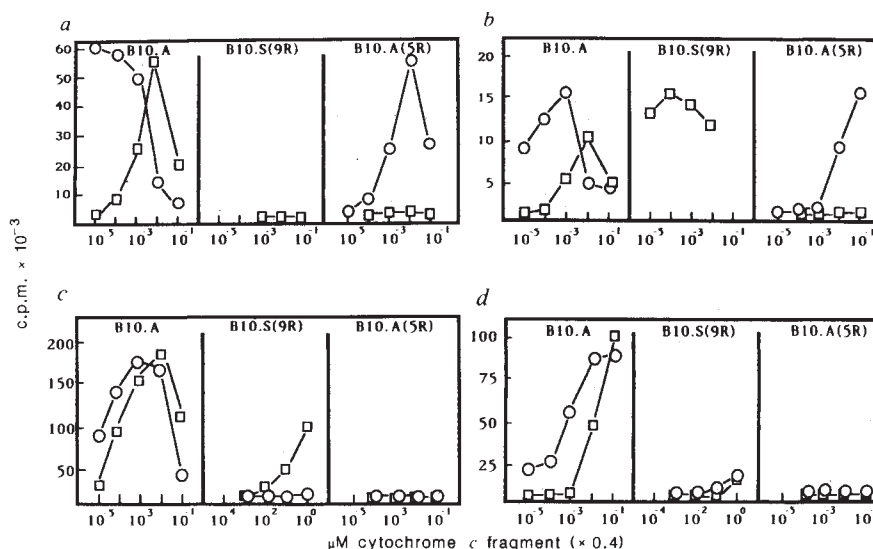
Cytochrome *c*-specific T-cell clones

T-cell lines, grown by antigen stimulation and cloned by limiting dilution, were used as uniform populations of immunocom-

petent T cells from which to clone TCR genes. Figure 2 shows the patterns of reactivity of four cytochrome *c*-specific T-cell clones, C.F6, 5C.C7, B10 and 4.C3, which originated from B10.A mice immunized *in vivo* with pigeon cytochrome *c*. Several characteristics of the B10.A anti-pigeon cytochrome *c* response are readily apparent from these data^{7-10,12-14,16,17}. First, in the presence of syngeneic B10.A antigen-presenting cells (APC), all four clones respond to lower concentrations of moth cytochrome *c* than to the immunogen, pigeon cytochrome *c*; this response has previously been termed heteroclitic^{7,8}. Second, there is a cross-reactivity in the specificity for MHC molecules: all four clones are able to respond to antigen presented by APC from more than one strain of mouse. As described more thoroughly elsewhere^{9,12-14}, the hierarchy of the responses to phylogenetic cytochrome *c* variants depends on the genotype of the APC. Third, one clone, 5C.C7, proliferates in the absence of cytochrome *c* in response to allogeneic B10.S(9R) and B10.S APC and therefore recognizes I-A^s in the absence of added antigen (see Fig. 2 legend)⁹. The characteristic phenotypes represented by the clones in Fig. 2 encompass most of the clonotypes that have been observed in the B10.A response to pigeon cytochrome *c*^{9,12,14}. The frequencies of the clones exhibiting each of these four phenotypes differ markedly. Approximately 85% of all pigeon cytochrome *c*-reactive B10.A T-cell clones exhibit a C.F6-like phenotype, including the T-cell hybridoma 2B4 (originally designated A-1 in ref. 12), with the rest of the clones resembling 5C.C7 and B10 (refs 9, 12). The phenotype of clone 4.C3 as a variant of the B10 phenotype can be distinguished only by a detailed analysis of fine specificity, therefore its frequency has not been determined.

Although all four clones differ from one another in their antigen dose-response curves and in the antigen fine specificity of their cytochrome *c* responses, they can be grouped into two pairs. C.F6 and 5C.C7 are very similar in their response to antigen on B10.A and on B10.A(5R) APC, except that C.F6 is more sensitive to stimulation with the moth fragment (Fig. 2). Neither C.F6 nor 5C.C7 responds to either pigeon or moth cytochrome *c* fragment on B10.S(9R) APC, even at concentrations 10-fold higher than those presented in Fig. 2 (24 μ M, data not shown). The most striking difference in specificity between clones C.F6 and 5C.C7 is the presence of alloreactivity to I-A^s in 5C.C7. The second pair of clones, B10 and 4.C3, have in common the phenotype of responding to cytochromes *c* in association with B10.A and B10.S(9R) APC, but, at the concentrations of antigen tested, not in association with B10.A(5R) APC. There are, however, significant differences in their fine specificity of antigen recognition. Most notably, B10 is 50-100-fold more sensitive to antigen (cytochrome *c* fragments 81-103/104) on B10.A APC than is 4.C3, and B10 does not respond to moth fragment on B10.S(9R) APC. In contrast, 4.C3 responds at least as well to moth fragment as to pigeon fragment

Fig. 2 Proliferation of four B10.A T-cell clones (*a*, C.F6; *b*, 5C.C7; *c*, B10; and *d*, 4.C3) in response to doses of the carboxy-terminal CNBr fragments of pigeon and moth cytochromes *c*. Clones were derived as described previously^{9,12} from B10.A lymph node cells primed once *in vivo* to pigeon cytochrome *c*, multiply stimulated *in vitro* with pigeon cytochrome *c* on B10.A APC (C.F6), and then selected by stimulation with antigen on B10.S(9R) APC (B10 and 4.C3) or on B10.S APC alone (5C.C7), and cloned by limiting dilution. 4.C3 and 5C.C7 were derived from the same line of T cells, while B10 and C.F6 were each derived independently. All four clones are L3T4⁺ Lyt2⁻. Values plotted are c.p.m. of ³H-thymidine incorporated in a 12–18-h pulse begun on day 3 of co-culture of 10⁴ responder cells with 5 × 10⁵ irradiated (3,300 rad) spleen cells as a source of APC. Pigeon fragment 81–104 (□) and moth fragment 81–103 (○) were added in the indicated doses at the beginning of the culture. APC were derived from B10.A (left-hand panels), B10.S(9R) (middle panels), or B10.A(5R) mice (right-hand panels). Neither C.F6 nor 5C.C7 responded to moth fragment on B10.S(9R) APC. Incorporation in the absence of cytochrome *c* ranged from 210 to 930 c.p.m., except for the 5C.C7 anti-B10.S(9R) response (14,000 c.p.m. incorporation). This alloreactivity manifest by clone 5C.C7 is seen in response to B10.S APC and thus maps to *I-A^s*. The T-cell hybridoma 2B4 has a phenotype indistinguishable from that of C.F6¹².



on B10.S(9R) APC. The latter difference is even more obvious when the whole cytochrome molecule, rather than the fragment, is used as antigen (data not shown).

To emphasize the difference between the first and second pairs of clones, the proliferative responses of the four clones to whole pigeon cytochrome are presented (Table 1). Clones C.F6 and 5C.C7 respond to pigeon cytochrome *c* in association with B10.A APC, but not at all in association with B10.S(9R) APC, even at the highest doses of antigen (24 μM). In contrast, clones B10 and 4.C3 respond to pigeon cytochrome *c* in association with B10.A APC, and to about the same extent in association with B10.S(9R) APC, although with B10.S(9R) APC the dose-response curves are shifted to 10-fold higher concentrations of antigen.

These reproducible similarities and differences in phenotype, and the fact that these clones are representative of the overall B10.A cytochrome *c* response (an observation confirmed by Southern blotting of cytochrome *c*-reactive T-cell lines; S. B. Sorger, I. I. Engel, P.J.F., S.M.H. and L.A.M., manuscript in preparation), led to their selection for molecular analysis.

cDNA sequencing

To determine the structural basis for the above differences in T-cell specificity, cDNA copies of expressed α - and β -chain messages were cloned, isolated and sequenced. cDNA libraries

were constructed as described in Fig. 3 legend, and 20,000–100,000 colonies were screened separately for hybridization to α and β probes. The frequency of colonies that rescreened positively with the α and β probes ranged from 0.07 to 0.15% and from 0.06 to 0.16%, respectively. We routinely selected the longest cDNA inserts from each library for analysis, and in each case the first clone sequenced was the result of transcription from a productively rearranged gene. The formal possibility exists that there is more than one productively rearranged gene in a single T-cell clone, although experiments using an allotypic anti-TCR antibody are consistent with allelic exclusion of V_{β} genes³³. Furthermore, as TCR genes rearrange according to the same rules that govern immunoglobulin gene rearrangements³⁴, we expect that the rules of allelic exclusion will apply also to the expression of both β - and α -chain genes. Nonetheless, we attempted to determine that the cDNA clones analysed were copies of the only complete TCR transcripts present in each T-cell clone.

To examine β -chain rearrangements, genomic Southern blots were run on *EcoRI*-digested DNA from the clones analysed. We constructed four single-copy genomic probes spanning $J_{\beta}1$ (1.3 kilobase (kb) *SphI*–*SacI* fragment) and $J_{\beta}2$ (1.2-kb *EcoRI*–*Clal* fragment), and from sequences upstream of $D_{\beta}1$ (1.2-kb *PstI* fragment) and $D_{\beta}2$ (0.8-kb *PvuII*–*EcoRI* fragment). All rearrangements to the $J_{\beta}1$ and $J_{\beta}2$ loci can be seen using the

Table 1 Proliferative response of T-cell clones to pigeon cytochrome *c*

μM cytochrome <i>c</i>	24	2.4	0.24	0.024	0.0024	0
B10.A APC						
C.F6	16.9 ± 3.0*	34.4 ± 3.0	51.2 ± 3.9	13.6 ± 0.8	0.9 ± 0.0	0.35 ± 0.1
5C.C7	1.0 ± 0.1	4.4 ± 0.1	11.3 ± 0.1	1.5 ± 0.1	0.3 ± 0.0	0.3 ± 0.0
B10	4.0 ± 1.4	86.4 ± 9.3	173.8 ± 12.4	136.1 ± 26.3	5.2 ± 2.5	0.7 ± 0.1
4.C3	6.3 ± 1.4	35.4 ± 6.4	78.3 ± 3.8	23.3 ± 0.1	0.9 ± 0.0	0.6 ± 0.0
B10.S(9R) APC						
C.F6	0.4 ± 0.0	0.5 ± 0.2	0.4 ± 0.2	0.5 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
5C.C7	3.4 ± 0.8	5.9 ± 0.2	5.3 ± 0.8	8.1 ± 1.4	7.8 ± 0.1	8.1 ± 3.0
B10	20.2 ± 0.3	162.8 ± 0.6	132.9 ± 10.1	2.7 ± 0.1	1.3 ± 0.3	0.4 ± 0.2
4.C3	47.0 ± 6.6	41.5 ± 1.1	23.0 ± 11.0	1.2 ± 0.7	0.8 ± 0.1	0.6 ± 0.1

Values are c.p.m. × 10⁻³, rounded to the nearest 100, ± s.e.m. (± 0.0 represents < 50 c.p.m.). Experiments were performed as described in Fig. 2 legend.

two *J* probes, and *D-J* rearrangements can be distinguished from *V-D-J* rearrangements by determining whether a rearranged band that hybridizes to a *J* probe also hybridizes to an upstream *D* probe. In the case of a *V-D-J* rearrangement involving the deletion of the intervening chromosomal segment, the *D*-region sequences will be deleted, and in the case where a *V-D-J* rearrangement results from a DNA inversion^{35,36}, the *D* probe will hybridize to a different-sized restriction fragment. From this analysis we found that clones C.F6 and B10 have only one β -chain *V-D-J* rearrangement; all of the other possible β loci have undergone *D-J* rearrangements (data not shown). Clone 5C.C7 has two *V-D-J* rearrangements, but in four cDNA clones analysed, only the one presented was found. Clone 4.C3 was not analysed in this manner, but is phenotypically similar to B10, and as presented below has exactly the same productive gene rearrangement. Six other independent T-cell clones with the same phenotype as B10 also show the same rearrangement (data not shown).

Owing to the distance spanning the J_α chromosomal region, α -chain rearrangements are more difficult to analyse. However, using a V_α 4.C3 probe, we found exactly one rearrangement on three different digests for all the clones presented. A second V_α probe hybridizing to a V_α family of more than eight members showed no rearrangements in any of the clones. Furthermore, in all the clones analysed, the same V_α gene segment is expressed (see below). Without testing every existing V_α gene, we cannot rigorously exclude another productive rearrangement. However, we believe the weight of evidence favours allelic exclusion for the expression of rearranging gene families such as those encoding immunoglobulin and the TCR, and suggests that we have analysed the only productively rearranged gene in each T-cell clone.

The sequences of the α - and β -chain genes from the four T-cell clones are shown in Fig. 3, together with the sequences for 2B4 (refs 34, 37), a B10.A cytochrome *c*-specific T-cell hybridoma, added for comparison. In keeping with the nomenclature used for immunoglobulin *V* regions, we have referred to the variable regions of α - and β -chain genes presented here by the cells from which they were first isolated. Notwithstanding the recent attempts by two groups to impose a numbering system on the variable regions^{38,39}, we have chosen to wait until the genes are ordered on the chromosome before substituting numbers for names.

As shown by the sequences listed in Fig. 3a, all five cytochrome *c*-specific TCRs use members of the same V_α gene family. In B10 congenic mice, genomic Southern blots reveal five cross-hybridizing *Hind*III bands, possibly indicating a V_α family of five or more members (data not shown). Both Southern blot analysis and a comparison with the two germline sequences available (M. Davis and R. Wallich, personal communication) reveal that C.F6, 5C.C7, B10 and 4.C3 all use the same germline V_α gene, which we have labelled V_α 2B4.2, while 2B4 uses a second member of the family, V_α 2B4.1. The variable region expressed by 2B4 differs from that expressed by the other four T cells by 11 nucleotides, resulting in six amino-acid substitutions (Fig. 3a). C.F6, B10 and 4.C3 all have identical J_α elements, that is, J_α 84 (ref. 40), and apart from junctional differences discussed more fully below, all sequences are germline. To our knowledge, the *J* region of 5C.C7, here termed J_α C7, has not been sequenced previously⁴⁰⁻⁴². Thus, three J_α regions are used by these five α -chain genes— J_α 84, J_α 2B4 and J_α C7.

The β -chain gene sequences, illustrated in Fig. 3b, fall into two groups. C.F6, 5C.C7 and 2B4 all use the same V_β gene (V_β 2B4), and all sequences are germline. The two nucleotide differences between the leader region of 2B4 and the regions of 5C.C7 and C.F6 are the same two differences noted previously⁴³, and may represent errors in the original 2B4 sequence. B10 and 4.C3 use a different V_β gene, called here V_β B10, which has not been previously identified^{38,39}. Although the germline sequence of V_β B10 is unknown, the two sequences are identical up to the

V-D junction, and so are likely to be germline. The J_β sequences have been identified: J_β 1.2 (C.F6 and 5C.C7), J_β 2.5 (2B4; ref. 34) and J_β 2.1 (B10 and 4.C3). These J_β 1.2 and J_β 2.5 sequences match the previously sequenced germline J_β s^{34,44,45}. Our J_β 2.1 sequence matches that of Malissen *et al.*⁴⁵, but differs by a single nucleotide in triplet 108 from the sequence of Chien *et al.*³⁴.

We found to our surprise that one cDNA clone, 5C.C7 β 1, included a 71-base pair (bp) insert between the *J* and *C* regions (sequence not shown). Although the *V*, *D* and *J* regions of this clone were all in-frame, the inserted sequence caused the *C*-region sequence to be read out of frame. This inserted sequence has been seen previously in a cDNA clone isolated from a library made from concanavalin A-stimulated spleen cells, and has been identified as part of the intron between the first *J* cluster and C_β 1 (J. Kavalier, S.M.H., Y.-H. Chien and M. Davis, unpublished observation). Insertion of this sequence evidently results from a cryptic exon within the *J-C* intron, and in the case of 5C.C7 β 1, results in a nonfunctional message. Three other full-length cDNA clones from 5C.C7 lacked this insert and therefore represent functional, in-frame, messages.

As is evident from Fig. 3, the differences between the α and β sequences using the same *V* and *J* components all lie near the junctions between these components; in fact, 9 out of 10 cDNA clones show junctional variation, and no two sequences are completely identical. To emphasize this point, in Fig. 4a the C.F6, B10 and 4.C3 α sequences are aligned with the germline sequences of V_α 2B4.2 and J_α 84 (ref. 40). 4.C3 and C.F6 have deleted and/or altered *J*-region nucleotides, while B10 clearly results from an 'error-free' joining of *V*- and *J*-region elements. These observations indicate that if a D_α region does exist for

Fig. 3 Nucleotide and predicted amino-acid sequences of the expressed α - and β -chain genes from the four cytochrome *c*-specific T-cell clones in Fig. 2. **a**, α -Chain gene sequences. Nucleotides are grouped in triplets; the corresponding amino acids are given above the nucleotide sequence. Residue numbering is according to ref. 37 from which the 2B4 sequence was taken for comparison. Only those nucleotides and residues that differ from the top line of sequence are given. Cysteine residues are circled and potential sites for *N*-linked glycosylation are enclosed in brackets. Gaps (denoted by dashes) have been introduced where necessary to align homologous sequences. Arrows mark the boundaries of the leader peptide and of the *V*, *J* and *C* regions; dots indicate where these boundaries are uncertain. Dashes are used to standardize the lengths of the *J* regions to illustrate the contribution to the variability in the first residue of the *C* region by the last nucleotide of the *J* region. **b**, β -Chain gene sequences. As for **a**, except that numbering is according to ref. 34, from which the 2B4 sequence was taken for comparison. Sequences are listed in two groups; the first includes C.F6, 5C.C7 and 2B4, and the second includes B10 and 4.C3. Within each group, only those nucleotides and amino acids that differ from the top line of sequence are given. The leader peptide and *V*, *D* and *J* regions are marked with arrows. Probable *N*-regions⁵⁸ are indicated by the gaps on either side of the *D* region. A potential site for *N*-linked glycosylation in the 2B4 sequence from residues 97-99 has been omitted to avoid overcrowding. All sequences were determined from both strands. A comparison of the amino-acid sequences of the two V_β genes demonstrates a 37% homology; this is not significantly different from the overall homologies seen between two V_β genes selected at random^{38,39,53}.

Methods. Forty-eight hours after antigen stimulation, cloned T cells were expanded for 3 days in the presence of 100 U ml⁻¹ of recombinant human interleukin-2 from *Escherichia coli*⁵⁹ (provided as a grant to L.A.M. from Cetus Corporation, Emeryville, California). Total RNA was extracted from lyophilized cell pellets⁶⁰ and enriched for poly(A)⁺ RNA over oligo(dT)-cellulose columns⁶¹. Synthesis of oligo(dT)-primed double-stranded cDNA was performed as described elsewhere⁶², with some modifications⁶³. Homopolymer dG tails⁶⁴ were added to cDNA that had been size-selected on Sepharose 4BCL for a modal length of 1,600-1,850 nucleotides. Tailed cDNA was annealed to the dC-tailed vector pUC9 and transformed according to Hanahan⁶⁵. Libraries were screened with ³²P-labelled nick-translated probes for C_β (86T5; ref. 26) and α (provided by Dr E. Palmer)³¹. The longest cDNA clones from each library were sequenced by the method of Maxam and Gilbert⁶⁶.

[illegible]

$J_\alpha 84$, its use is not compulsory. 5C.C7 was not included in this comparison, as the germline sequence of $J_\alpha C7$ is unknown. In Fig. 4b, alignment of the β sequences from C.F6 and 5C.C7 with the germline sequences of $V_\beta 2B4$ (ref. 34), $D_\beta 1.1$ (refs 46, 47) and $J_\beta 1.2$ (ref. 44) reveals three to four N -region nucleotide insertions in each. Both C.F6 and 5C.C7 D -region sequences are read in the same frame. Figure 4b also shows a comparison of the B10 and 4.C3 sequences with their germline elements $D_\beta 1.1$ and $J_\beta 2.1$ (ref. 45), and with the consensus cDNA sequence for $V_\beta B10$. Again, junctional variability results from deletions and/or N -region nucleotide insertions. Figure 4b also illustrates that there are several ' N '-region nucleotides in common between C.F6 and 5C.C7 and between B10 and 4.C3. Because these clones were separately derived (Fig. 2 legend), this conservation of nucleotides may result from antigen selection or the common use of as yet undescribed D_β regions.

Discussion

Here we have analysed the TCR genes expressed in phenotypically related T-cell clones, and these data, shown schematically in Fig. 5, reveal for the first time several characteristics guiding the receptor specificity for antigen and MHC molecules.

Germline diversity. Clearly, the full range of I-E-restricted cytochrome *c* responses we see can be derived from the germline sequences of two members of a single V_α gene family. In fact, all 16 of the cytochrome *c*-specific T-cell clones of B10.A origin that we have examined (including those analysed on Southern gels) use some member of this V_α gene family. Furthermore, most of these T-cell clones use one of two different V_β genes, $V_\beta 2B4$ and $V_\beta B10$. Each individual α - and β -chain V region rearranges to one, or at most two different J regions in a large number of independent clones analysed (discussed below). Thus, these data indicate that only a limited number of the available α - and β -chain gene elements are expressed in the T-cell response to pigeon cytochrome *c* in B10.A mice. This limited germline diversity suggests that both chains contribute to the overall specificity of this T-cell response (see below).

Somatic mutation. In analysing these gene sequences we found that all V_α and $V_\beta 2B4$ sequences are germline, and the two $V_\beta B10$ sequences do not differ from one another. Thus, despite the fact that frequent junctional variation compared with the germline sequence can affect the 'affinity' of the receptor-ligand interaction (see below), we have observed no evidence for somatic mutation within the variable regions. This finding contrasts sharply with the well-documented hypermutation in the V regions of antibodies raised during secondary immune responses^{48,49}. However, these two systems are not entirely comparable as our T cells were primed *in vivo* and repeatedly stimulated with optimal concentrations of antigen *in vitro*, while the B-cell responses studied were allowed to mature *in vivo*.

Junctional diversity. The TCR genes of both B10 and 4.C3 are composed of the same genetic elements, differing only at the junction of the gene rearrangements (Fig. 4). Therefore, the 50–100-fold difference in response to a given dose of the antigen/MHC ligand, and the difference in response to moth cytochrome *c* in the context of I-E^s (Fig. 2), both probably result from this junctional variation. The caveat to this interpretation is that there could be variation in the number of receptors expressed by the two T-cell clones. This appears not to be the case because the level of expression of the α - and β -chain messenger RNAs appears to be comparable in B10 and 4.C3 (by cDNA clone frequency, and by the degree of hybridization on RNA blots), and the large dose-response difference for the two clones is not seen when whole cytochromes *c* are tested instead of the carboxy-terminal peptides (Table 1). Nonconservative changes that may cause a significant structural alteration occur in the α -chain (B10-Ala $\rightarrow$ 4.C3-Ser) and in the β -chain (B10-Asn $\rightarrow$ 4.C3-Val). This comparison between B10 and 4.C3 TCR sequences illustrates that junctional variability can alter antigen reactivity, and is thus selectable by antigen

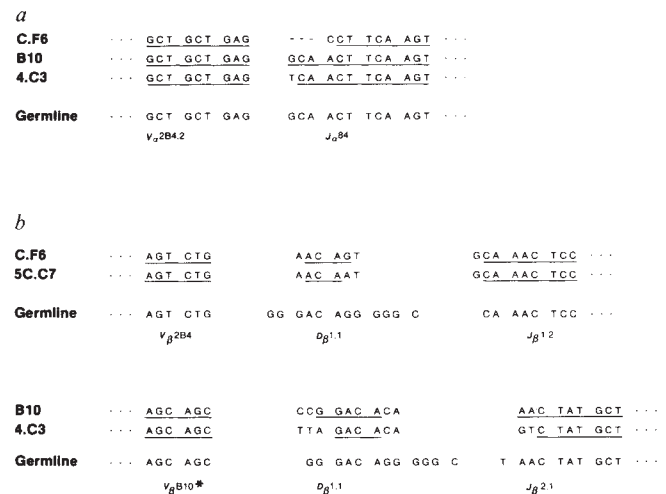


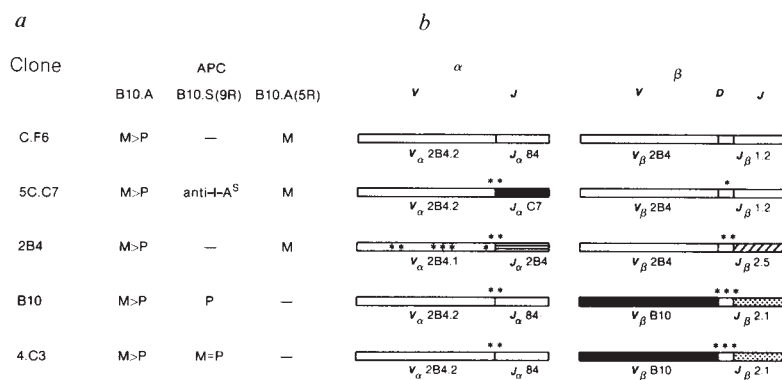
Fig. 4 Junctional variability in TCR genes. *a*, α -Chain gene nucleotide sequences from C.F6, B10 and 4.C3 are aligned with germline sequences from $V_\alpha 2B4.2$ (provided by M. Davis and R. Wallich) and $J_\alpha 84$ (ref. 40). The nucleotides in the cDNA sequences which correspond to those of the germline sequences are underlined. The sequence from 5C.C7 is not included because the germline sequence for $J_\alpha C7$ is unknown^{40–42}. *b*, β -Chain gene nucleotide sequences from C.F6 and 5C.C7 compared with the germline sequences of $V_\beta 2B4$ (ref. 34), $D_\beta 1.1$ (refs 46, 47) and $J_\beta 1.2$ (ref. 44). Sequences from B10 and 4.C3 are aligned with the known germline sequences of $D_\beta 1.1$ and $J_\beta 2.1$ (refs 34, 44). The germline sequence of $V_\beta B10$ is unknown; for the sake of comparison, the consensus cDNA sequence is listed. Nucleotides from the cDNA clones corresponding to those in the germline sequences are underlined.

during an immune response. Furthermore, the occurrence of junctional differences around D_β suggests the importance of this region of the receptor in ligand recognition. The fact that β -chain D regions are read in every frame³⁸ indicates that rather than being unselected, D_β may also be an important element in generating TCR diversity. We note that the D regions in the five sequences presented here are all read in the same frame. **Combinatorial joining.** A comparison between the sequences of the 5C.C7 and C.F6 TCRs should reveal the origin of the alloreactivity in 5C.C7; this is of general interest as such cross-reactivities for allogeneic MHC molecules are often seen in MHC-restricted T-cell populations^{50,51}. Barring the possibility of an independent alloreactive receptor (a possibility disfavoured by the available evidence⁵²), our structural data suggest that the anti-I-A^s reactivity of 5C.C7 is the result of the substitution of $J_\alpha C7$ into the other gene elements making up the receptor expressed by C.F6. Despite this alternative J usage, the two clones exhibit very similar antigen/MHC specificity. Similarly, C.F6 and 2B4 use different J regions in both the α - and β -chains, yet the specificity for antigen/MHC molecules is not affected appreciably.

On the basis of this analysis, it seems that different portions of the TCR heterodimer can contribute to combining sites, so that a change in the J_α of the receptor, although not significantly affecting cytochrome *c*-plus-MHC specificity, could create a combining site of sufficient affinity for I-A^s-encoded molecules to reveal an alloreactivity. This could correlate with the absence of three or four distinct regions of hypervariability in the α - and β -chain sequences compared with immunoglobulin variable regions^{37–39,41,53}. Possible complementarity-determining regions of the TCR might span much more of the primary sequence of the V , D and J regions than in immunoglobulin.

Combinatorial association. Perhaps the most striking observation arising from our data is the correlation of the expression of V_β

Fig. 5 Correlation of receptor gene expression with T-cell phenotype. *a*, Summary of reactivity patterns for the cytochrome *c*-reactive T-cell clones in Fig. 2. M refers to cytochrome *c* from tobacco hornworm moth; P refers to pigeon cytochrome *c*. *b*, Summary of receptor genes expressed by the various T-cell clones. The elements comprising the expressed α - and β -chain genes are distinguished by different patterns. All α -chain genes use one of two members of the $V_{\alpha}2B4$ family. $V_{\alpha}2B4.1$ differs from $V_{\alpha}2B4.2$ by six amino acids, indicated by the asterisks within the V region. All five cDNA V-region sequences are germline. Other asterisks represent junctional amino-acid differences compared with the C.F6 sequence, summarized from Fig. 4.



with a clonal phenotype—B10 and 4.C3 express $V_{\beta}B10$ and react with antigen and I-E^k _{β} or I-E^b _{β} but not I-E^b _{β} , and 2B4, C.F6 and 5C.C7 express $V_{\beta}2B4$ and recognize antigen plus I-E^k _{β} or I-E^b _{β} but not I-E^b _{β} . With the small variations already noted, all clones express the same α -chain. This pattern of function and TCR expression holds for all 16 independently derived T-cell clones examined on Southern blots using V_{α} and V_{β} probes. We conclude that in association with homologous $V_{\alpha}J_{\alpha}$ sequences, the sequence differences between $V_{\beta}2B4$ – $J_{\beta}1.2$ and $V_{\beta}B10$ – $J_{\beta}2.1$ change the overall conformation of the T-cell receptor such that the specificity for MHC molecules in association with cytochrome *c* is altered. However, the data presented here do not provide evidence for the physical association of one or another chain with antigen and MHC molecules, and as discussed above, variations in the β -chain can affect antigen fine specificity, and variations in the α -chain may affect allogeneic MHC specificities. As described above, both α - and β -chains appear to be selected in the B10.A response to pigeon cytochrome *c*, therefore both may contribute to the antigen/MHC specificity. For comparison, in the case of immunoglobulins, the antigen specificity has been variously shown to be most closely associated with either the heavy chain, the light chain, or both^{48,49}, yet presumably both chains are necessary for the overall antigen-antibody interaction. Future studies will determine whether all T-cell responses are selected on the basis of both α - and β -chains, or whether, like immunoglobulin, some responses depend mainly on the structure of one chain or the other.

Although changes in one chain can apparently alter the specificity for MHC molecules without appreciably affecting the specificity for antigen, the combining site is likely to manifest a specificity for antigen and MHC molecules that is not predictable based on the sequence of either chain alone. This concept is underscored by comparing the β -chain sequence from a T-cell clone presented here (C.F6), which is specific for cytochrome *c* and I-E^{k/b}, with that of clone 3H.25, which is specific for chicken egg lysozyme and I-A^b (ref. 43). These two clones express the same $V_{\beta}2B4$ – $J_{\beta}1.2$ combination, the only difference being that the *D* region is read in a different frame, yet they do not appear to have any specificity characteristics in common.

The mechanisms for generating diversity in immunoglobulins include junctional diversity, combinatorial V–(D)–J joining, combinatorial association of the heterodimer heavy and light chains, and also somatic mutation^{48,49,54,55}. As the structure of the TCR α - and β -chain genes is analogous to that of immunoglobulins, the mechanisms for generating diversity in the latter were proposed to contribute to the T-cell repertoire also. Here we have shown that, with the exception of somatic mutation, each of the mechanisms that generate diversity in immunoglobulins has clear effects on the specificity of T cells and therefore the T-cell repertoire.

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LETTERS TO NATURE

Weakly interacting massive particles and solar oscillations

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If the Sun were to contain even a minute mass fraction of weakly interacting massive particles (WIMPs), there could be a significant influence on its central thermal structure. In particular, a relative concentration as small as $\sim 10^{-11}$ by number may lower the central temperature sufficiently to bring the predicted electron neutrino detection rate into agreement with observation^{1–6}. Helioseismology^{7,8} provides a means for an independent test of the validity of this and other proposed resolutions of the solar neutrino problem. Theoretically, it is the low-degree g modes that are most sensitive to conditions in the core, the only region where substantial deviations from so-called standard solar models occur. Indeed, solar models with WIMPs have a g-mode period spacing that is markedly different from that of other solar models. Therefore g-mode observations hold the promise of a sensitive test, although unfortunately their current interpretation is fraught with difficulties. The best test currently available involves instead the frequency separation of low-degree p modes with like $(n + \frac{1}{2}l)$ (where n and l are respectively the order and degree of the mode). Standard solar models produce p-mode separations somewhat larger than those observed. Conventional attempts to resolve the solar neutrino problem^{9,10} make the situation worse; in some cases, grossly so. We show that, in contrast, a relevant WIMP model predicts p-mode separations that are reduced by $\sim 10\%$; this is consistent with the observations.

The main effects of WIMPs on solar models are as follows². By virtue of their long mean-free-paths, WIMPs transport energy readily, and tend to produce an isothermal core. We emphasize that this is a consequence of the changed energy transport, and occurs without any mixing of the conventional baryonic component. Thus the relative molecular-weight gradient built up during evolution is not destroyed. The central temperature, T_c , is reduced, leading to a greatly reduced solar neutrino flux. In particular, a WIMP-accreting solar model³ that has a current relative number concentration $N_c \approx 1.6 \times 10^{-11}$ of 5-GeV WIMPs reduces the temperature at radii $r \leq 0.07 R_\odot$, lowering T_c from $\sim 15.1 \times 10^6$ to $\sim 13.2 \times 10^6$ K, and produces a neutrino counting rate of 2.02 s.n.u. (1 s.n.u. = 10^{-36} captures per second per ^{37}Cl atom³⁷), consistent with the result $(2.1 \pm 0.3 \text{ snu})$ obtained with Davis's ^{37}Cl detector¹¹. As already mentioned, the core is essentially isothermal. However, in order to obtain the observed value for the luminosity, $L_\odot$, temperatures are slightly higher immediately outside this core than they are in conventional solar models, the difference decaying to a negligible amount for $M_r \geq 0.25 M_\odot$ ($r \geq 0.16 R_\odot$), where M_r is the mass enclosed in a sphere of radius r concentric with the Sun.

The reduction in T_c has two main consequences: (1) since central pressure support must be maintained, the central density

ρ_c is increased (from ~ 166 to $\sim 196 \text{ g cm}^{-3}$ in the models of ref. 2), and (2) since less hydrogen is burnt at the centre of the core, the central hydrogen abundance X_c is somewhat larger now than it is in conventional models (~ 0.48 rather than ~ 0.39). The increased values of ρ_c and X_c partially offset the effect of the lowered T_c on central energy production. Minor changes are required to the initial hydrogen abundance and the mixing-length parameter to produce a solar model with the observed luminosity and radius at the presumed age of the Sun ($4.7 \times 10^9 \text{ yr}$).

This and other models of the Sun may be subjected to seismological tests. The pulsation modes with frequencies most sensitive to conditions in the core are the internal g modes. Unfortunately, unambiguous measurements of such modes are not yet available^{8,12}; we must therefore be content with using the low-degree p modes, for which excellent data exist^{13–16}. Only p modes of low degree penetrate to the core, and even for these, the core makes only a small contribution to the frequencies. We use, as a diagnostic, the frequency separation

$$\Delta_{n,l} \equiv \nu_{n,l} - \nu_{n-1,l+2} \quad (1)$$

of 5-min modes with cyclic frequencies $\nu_{n,l}$ which, for low degree l , is relatively insensitive to the uncertain structure of the outer convective boundary layer and is influenced by conditions in the core¹⁷.

Existing observations and theoretical results are summarized in Table 1. The standard theoretical treatments have usually derived $\Delta_{n,l}$ (or some suitable average for different, closely grouped modes) by differencing numerically computed eigenfrequencies of modes with appropriate n and l from the chosen solar models. Such approaches have produced frequency separations for standard solar models that are not significantly different from observation: as recorded in Table 1, the mean theoretical value of $\Delta_{n,0}$, which is the more sensitive measure of conditions in the core because the lower-degree modes penetrate more deeply, is only 3–9% greater than the mean separation obtained from the observations. The situation for previous models with reduced neutrino fluxes is much worse. Among such models, those currently most widely favoured have either (1) a low initial helium abundance Y_0 (refs 18–21), for example $Y_0 \approx 0.17$, or (2) diffusively mixed cores, as suggested by Schatzman *et al.*¹⁰. However, both types of model give frequency separations $\Delta_{n,l}$ that are substantially greater than those of conventional standard models¹⁷. Furthermore, models with low Y_0 have high-degree p-mode frequencies that are inconsistent with observation¹⁷.

An alternative method of estimating the frequency separation of p modes is provided by asymptotic analysis²². Acoustic modes that penetrate to the core have $n \gg l$, and for these $\Delta_{n,l}$ is given by

$$\Delta_{n,l} \approx \frac{(2l+3)\nu_0}{2\pi^2\nu_{n,l}} \left[\frac{c(R_\odot)}{R_\odot} - \int_0^{R_\odot} \frac{1}{r} \frac{dc}{dr} dr \right] \quad (2)$$

as $n/l \rightarrow \infty$, where $c(r)$ is the local sound speed and

$$\nu_0 = \left(2 \int_0^{R_\odot} \frac{dr}{c} \right)^{-1} \quad (3)$$

Because there exist higher-order corrections to equation (2) which may not be negligible for the finite values of n/l associated with the observations, one cannot rely on the absolute value of

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$\Delta_{n,l}$ for checking solar models. This is evident in Table 1, where one sees that the mean normalized frequency separation Δ_b , defined by equation (8) (see below), varies with l . (For Christensen-Dalsgaard's²³ solar model 1, the asymptotic expression (2) yields $\Delta_l \approx 10.4 \mu\text{Hz}$.) Nevertheless, the ratio of the asymptotic values of $\Delta_{n,l}$ for two slightly different solar models (with only minor modifications to otherwise common assumptions) does provide a good estimate of the actual ratio. We have therefore compared the asymptotic separations for both standard and WIMP models.

The primary data for this comparison were taken from the solar evolutionary models of Faulkner and Gilliland²; specifically, standard model A and the WIMP model C of that paper. We did not have immediate access to the detailed numerical results of that work, and therefore resorted to reading data from Figs 1 and 2 of ref. 2. We judge that by this technique we could obtain the temperature $T(M_r)$ and the density $\rho(T)$ to three-figure accuracy except, in model C, where the core is essentially isothermal and thus $\rho(T)$ is extremely steep. We obtained $\rho(T)$ in the latter case, and $r(M_r)$ for both models, by integrating the continuity and pressure-support equations. This necessitates a knowledge of the equation of state, $p = p(\rho, T, X)$, where p is pressure, and of the variation of the hydrogen abundance $X(M_r)$. The former was obtained by an approximation described in ref. 24. The gas pressure p_g comprises separate contributions from the nuclei and the electrons such that

$$p_g = \frac{R}{\mu_n} \rho T + p_e \quad (4)$$

and

$$p_e = \frac{R}{\mu_e} \rho_{\text{id}} T = K_1 \left(\frac{\rho_{\text{deg}}}{\mu_e} \right)^{5/3} \quad (5)$$

where R is the ideal gas constant, μ_n and μ_e are the mean relative molecular weights of the nuclei and the electrons respectively, and $K_1 = (h^2/20m_e m_H)(3/\pi m_H)^{2/3} = 9.91 \times 10^{12}$ c.g.s. units is Chandrasekhar's nonrelativistic degeneracy parameter, where h is Planck's constant, and m_e and m_H are the electron and proton masses. The purely formal 'densities' ρ_{id} and ρ_{deg} defined by equation (5) yield the actual density ρ in equation (4):

$$\rho = (\rho_{\text{id}}^{-2} + \rho_{\text{deg}}^{-2})^{-1/2} \quad (6)$$

Equations (4), (5) and (6) may be manipulated to produce a form for $p_g(\rho, T, X)$ which can easily be solved iteratively for $\rho(p_g, T, X)$. The 10% contributions from partial degeneracy can themselves be represented to $\sim 3\%$ accuracy.

Reference 2 does not provide $X(M_r)$, but it does give the present central values, namely $X_{\text{cA}} = 0.395$, $X_{\text{cC}} = 0.477$ (for models A and C respectively). To obtain $X(M_r)$ we resorted to a modification of the information concerning $X(M_r)$ given in Fig. 7 of a related, updated paper³. In that paper, the standard model (1) has a central hydrogen abundance $X_{\text{c1}} = 0.379$ and the WIMP model (3) has $X_{\text{c3}} = 0.450$. Of greater relevance in the present context (estimation of $X(M_r)$ for the earlier models²) are the amounts of hydrogen burnt. At the centre these are $\Delta X_{\text{cA}} = 0.342$, $\Delta X_{\text{cC}} = 0.268$, $\Delta X_{\text{c1}} = 0.349$ and $\Delta X_{\text{c3}} = 0.285$. The amounts for the standard models are substantially the same, and we obtained $X_A(M_r)$ by adding to $X_1(M_r)$ the difference in central amounts burnt; that is, 0.007. This should give $X_A(M_r)$ in the important central regions to sufficient accuracy. For $X_C(M_r)$ the adjustment requires more care. The WIMP models of refs 2 and 3 were computed subject to slightly different assumptions: for ref. 2 the WIMPs have mass 4 GeV, they are present throughout the history of the Sun, and the energy exchange between WIMPs and baryonic material is treated rather crudely; for ref. 3, the WIMPs have mass 5 GeV, they are accreted linearly in time, and the energy exchange is according to the Spergel and Press⁴ treatment. Because more of the hydrogen is consumed at later stages when the luminosity is

Table 1 Mean normalized p-mode frequency separations

	$\bar{\Delta}_0(\mu\text{Hz})$	$\bar{\Delta}_1(\mu\text{Hz})$	Ref.
Observations			
	8.6	—	13
	10.0	9.8	14
	9.0	—	15
	—	9.7	16
All	9.2 ± 0.6	9.7 ± 0.03	
Standard solar models			
	10.1	9.7	21
	10.5	9.5	23
	10.4	7.4	35
	6.6	7.2	35
	9.3	9.6	27
	10.1	10.4	36
	—	11.8	33
All	10.0 ± 0.4 (9.5 ± 1.4)	10.2 ± 0.8 (9.4 ± 1.5)	
Diffusively mixed models			
	15.4	12.2	27
	16.8	17.0	31
	13.4	12.2	32
	—	13.7	33
Helium-deficient model			
	13.2	10.3	21

$\bar{\Delta}_l$ are averages of the available normalized separations Δ_b , defined by equation (8), over the frequency range 2.0–4.0 mHz (excluding the separation involving p_{13} ($l=4$) of the standard model of ref. 27, the frequency of which appears to have been misprinted). The standard solar models all have conventional compositions ($Y_0 \approx 0.25$, $Z \approx 0.018$); the helium-deficient model had $Y_0 = 0.16$. Ulrich and Rhodes²⁷ report frequency separations of a model with 0.3 of the heavy-element abundance Z of their standard; if their standard value is assumed to be 0.018 and if their results are extrapolated linearly in $\log_{10} Z$ to -3.0 (the value corresponding to $Y_0 = 0.16$ in ref. 21), the separations obtained are similar to those of ref. 21. Ulrich and Rhodes³⁶ report differences between their theoretical eigenfrequencies and the observed frequencies quoted by Grec *et al.*¹⁴, from which we have deduced the theoretical eigenfrequencies; differences for modes for which Grec *et al.* do not quote frequencies were ignored. The rows labelled 'All' record the means and standard deviations of the entries immediately above. The values from ref. 35 have been omitted from the main averages of the standard solar models (they are included in the entries in parentheses immediately following) because the marked difference between the values of $\bar{\Delta}_0$ derived from the two models with quite similar characteristics (they differ only in the values of their initial compositions, which are $Y_0 = 0.233$, $Z = 0.020$ for the first model and $Y_0 = 0.229$, $Z = 0.018$ for the second) suggests that the very central regions of the models might have been inaccurately computed. The diffusively mixed model of ref. 27 was computed using the more highly mixed (with effective Reynolds number $\text{Re}^* = 200$) composition profile of ref. 10; the other diffusively mixed models are for the model with the lesser degree of mixing (with effective Reynolds number $\text{Re}^* = 100$), which reproduces the neutrino flux. Berthomieu *et al.*³¹ estimated Δ_l by asymptotic analysis and reported it to be 5/3 of their standard value; the values quoted above are accordingly 5/3 of the mean theoretical values of the standard solar models quoted higher in the table. Christensen-Dalsgaard³² estimates a single value of Δ_l for each solar model by fitting eigenfrequencies to Tassoul's formula for $0 \leq l \leq 3$ and a frequency range similar to that used here, the ratio for the mixed and standard models being 1.28; the values quoted above were obtained by scaling the values for Christensen-Dalsgaard's standard model listed higher in the table by that ratio.

greater, and because the luminosity is constrained to have the same value now, the integral over M_r of hydrogen consumed must be essentially the same for all models. Thus $X_C(M_r)$ was obtained by first considering the difference between the amounts of hydrogen consumed in the WIMP and standard models to be divided into two parts: a contribution from the final WIMP concentration and, in the case of model 3 of ref. 3, a contribution from the difference between the WIMP concentration at any instant and its final value; it was then assumed that the former

contribution is the same in model C and model 3. Thus we adopted for $X_C(M_r)$ the linear combination:

$$X_C = X_3 + (X_{CC} - X_{C3}) + \lambda(X_1 - X_3 - \varepsilon) \quad (7)$$

with $\varepsilon = 0.011$ chosen to render the integral with respect to M_r of the third term on the right-hand side equal to zero, and $\lambda = 0.27$ chosen to make X_{CC} agree with the value reported in ref. 2.

The hydrostatic equations were integrated using, in addition to p , 'natural' Faulkner variables²⁵, r^2 and $M_r^{2/3}$, which have a desirable linear relation in the central regions. The integrations produced an excellent fit (again, to the measurable three-figure accuracy) to $\rho(T)$ of the WIMP model out to the region ($M_r \approx 0.09 M_\odot$) where $\rho(T)$ attained a moderate slope and the relationship could be read to the desired accuracy from Fig. 2 of ref. 2. The appropriateness of the procedure we have adopted can be judged from this fit, and from the fact that a similar integration using only the ideal gas law and $X_3(M_r)$ for $X_C(M_r)$ was badly in error in this region.

Once state variables had been determined as functions of M_r , or equivalently r , the velocity of sound was determined from $c^2 = \gamma p / \rho$ with the assumption $\gamma = 5/3$. It was then possible to evaluate the integral in equation (2) out to $M_r = 0.25 M_\odot$ ($r \approx 0.16 R_\odot$), the limit of the values in ref. 2. Beyond that point, conditions in the WIMP model have reverted essentially to those of the standard model. The evaluation of $\int dc/r$ in the vicinity of $r=0$ was carried out using a locally determined series expansion for $c(r)$ up to $O(r^4)$. The integrals, which we evaluated to $r = r_1 = 0.16 R_\odot$, encompass two approximately cancelling regions of rising and falling sound speed; they have values $I_A(r_1) = -1.09 \times 10^{-4} \text{ s}^{-1}$ (standard) and $I_C(r_1) = +2.01 \times 10^{-5} \text{ s}^{-1}$ (WIMP), and the integrands at $r = r_1$ are the same. The remainder of the integrals, from $r = r_1$ out to the surface, were estimated from the $c(r)$ relation reported in ref. 26 for the standard solar model of ref. 23. The final estimates are $I_A(R_\odot) = -1.54 \times 10^{-3} \text{ s}^{-1}$ and $I_C(R_\odot) = -1.41 \times 10^{-3} \text{ s}^{-1}$.

It is convenient to represent the results in terms of the normalized frequency separation

$$\Delta_l = \frac{3\nu_{n,l} \Delta_{n,l}}{(2l+3) \nu_s} \quad (8)$$

where ν_s is simply a scaling factor which we have chosen to be 3.0 mHz in order to make the value of Δ_l typical of the separations $\Delta_{n,l}$ with $l=0$. According to equation (2), Δ_l should be independent of l and n . Taking $c(R_\odot) = 0.008 \text{ Mm s}^{-1}$ we obtain the asymptotic ratio

$$\frac{\Delta_l(\text{WIMP})}{\Delta_l(\text{standard})} = \frac{c(R_\odot)/R_\odot - I_C(R_\odot)}{c(R_\odot)/R_\odot - I_A(R_\odot)} = 0.92 \quad (9)$$

which we believe is more reliable than the individual asymptotic values of Δ_l . (Those values, when computed with $\nu_0 = 139 \text{ } \mu\text{Hz}$ (ref. 16), are 10.9 μHz and 10.0 μHz for the standard and WIMP models respectively). We adopt, however, $\Delta_l(\text{standard}) = 10.0 \text{ } \mu\text{Hz}$ (the mean value for $l=0$ from Table 1) in the frequency range of the observations, and therefore infer

$$\Delta_l(\text{WIMP}) \approx 9.2 \text{ } \mu\text{Hz} \quad (10)$$

This result is in excellent agreement with the observations.

We recognize that the analysis is approximate and that detailed numerical eigenfrequency computations will be required to confirm the result. Moreover, the uncertainty in current theoretical estimates from standard models, and in the observations themselves, renders this otherwise impressive agreement weak. Nevertheless, neither the separation Δ_l of model C nor that of the standard model deviates significantly from the observations. The important point is that the WIMP model is the only model known to be in agreement with both the solar neutrino and the oscillation measurements.

Although the value of ν_0 is a relatively poor indicator of the structure of the core, because it is most sensitive to conditions

in the outer layers of the Sun where the sound speed is comparatively low, it is nonetheless interesting to note how it is influenced by WIMPs. By evaluating the integral in equation (3) for models A and C out to $r = r_1$, and using the value appropriate to model 1 of ref. 23 beyond r_1 , we find that the presence of WIMPs increases ν_0 by $\approx 0.05\%$. This is to be compared with adjusting the initial helium abundance, which causes a reduction in ν_0 by about 5% if Y_0 is reduced from 0.25 to 0.15 (ref. 21), and with extensive diffusive mixing, which, like the WIMPs, probably has quite a small effect²⁷.

As mentioned previously, the g modes are more sensitive to the structure of the core. Even though unambiguous measurements are not yet available, we can estimate the implications of the WIMP models. The asymptotic normalized period spacing $\Delta P = [l(l+1)]^{1/2} (\nu_{n+1,l}^{-1} - \nu_{n,l}^{-1})$ of high-order g modes of low degree is given by^{22,28,29}

$$\Delta P \approx 2\pi^2 \left(\int_0^{r_c} r^{-1} N dr \right)^{-1} \quad (11)$$

as $n/l \rightarrow \infty$, where N is the buoyancy frequency:

$$N = \frac{g}{c} \left(-1 - \frac{c^2}{g\rho} \frac{d\rho}{dr} \right)^{1/2} \quad (12)$$

$g = GM_r/r^2$ is the local gravitational acceleration and r_c is the radius of the base of the convection zone. As for the other estimates, we evaluated the integral in equation (11) out to $r = r_1$ for the standard and WIMP models A and C, and the contribution to the remainder, about 35% of the total, was taken from Christensen-Dalsgaard's²³ standard model 1, the buoyancy frequency of which is plotted in ref. 30. The resulting values of ΔP for the standard and WIMP models were 38 min and 31 min respectively. In view of the circuitous method by which we have made the estimates, which required differencing values that were read from possibly inaccurately drawn graphs, we cannot rely on the absolute values of the period spacing. However, in the belief that there may be some cancellation of the systematic errors in our procedure, we conjecture, as for our estimation of $\Delta_{n,l}$, that the ratio of the two values (0.82) is more accurately represented. Adopting ΔP (standard) = 36 min, the average of the values obtained in refs 31-33, we thus infer that ΔP (WIMP) ≈ 29 min. In marked contrast, the mixed models after Schatzman and Maeder¹⁰ that reproduce the neutrino flux have $\Delta P \approx 56 \pm 2 \text{ min}$ ³¹⁻³³, $\sim 40\%$ greater than in the corresponding standard model, and a model with $Y_0 = 0.16$ has a value $\sim 4\%$ greater than that in a standard model with $Y_0 = 0.25$ (ref. 21).

In both the WIMP models^{2,3} and the Schatzman-Maeder mixed models¹⁰, a lowered value of T_c is responsible for removing the solar neutrino discrepancy. It is therefore worth understanding in general terms why their predictions for the p -mode separations are so very different. The integral in equation (2), $I = \int dc/r$, is negative in standard models. However, since $c^2 \approx \gamma RT/\mu$ (neglecting the small degeneracy contribution to pressure), we see that because T varies slowly with r , and μ decreases more strongly from the centre (due to nuclear burning), c initially increases from the centre. Thus the central regions make a positive contribution to the integral in the standard model. When the central temperature is reduced (by δT_c) by homogenizing the material, as in the Schatzman-Maeder case, μ is also reduced, and varies much more gradually in the central regions. Indeed, $\delta\mu_c/\mu_c \approx 3\delta T_c/T_c$, and therefore the sound speed at the centre is higher than it is in the standard model. In the models considered by Schatzman *et al.*¹⁰, that change is so large that c actually attains its maximum value at $r=0$. Thus the central regions no longer provide a positive contribution to the integral I . The total integral, and hence Δ_l , acquires a larger absolute value.

On the other hand, in the WIMP models T_c is reduced without destroying the μ gradient, as emphasized in the introduction. The lessened variation in the nuclear burning decreases the μ gradient somewhat. However, the increase in $c(r)$ is now sustained for a substantially greater distance from the centre, caus-

ing the central contribution to the integral to be larger. Thus the absolute value of the integral is reduced in magnitude, and Δ_I is smaller than in the standard models. In fact, if one accepts that the observed values of Δ_I are smaller than in standard models, and that the integral I is adjustable mainly in the central regions since there are fewer uncertainties further out, one is virtually compelled to maintain the positive value of dc/dr near the centre where r^{-1} is large. This precludes extensive material mixing. Any effective energy-transport mechanism that can reduce T_c without violating this constraint might in principle resolve the solar neutrino problem; transport by WIMPs is the only process that has been shown to do so.

Similarly, the g-mode results differ in the two cases. The central contributions to $\int r^{-1} N dr$ can be crudely approximated by

$$\frac{4\pi G}{3} \int \frac{\bar{\rho}}{c} \left(-1 + \frac{\gamma m}{m+1} \right)^{1/2} dr \quad (13)$$

where $\bar{\rho}(r)$ is the mean density of the material within the sphere of radius r and m is the central polytropic index; $m \approx 3$ in the standard model, and is extremely large in the almost isothermal core of the WIMP model. Thus, in a small central region, most important in determining the value of the period separation, the relative contributions satisfy

$$\frac{N(\text{WIMP})}{N(\text{standard})} \approx \frac{\rho_c(\text{WIMP})}{\rho_c(\text{standard})} \sqrt{\frac{8}{3}} \approx 1.9 \quad (14)$$

because the central sound speeds of the two models differ by only 2%. Thus there is evidently a much larger contribution to the integral in the WIMP model. The Schatzman-Maeder model again produces a change of the opposite sign, mainly because chemical homogenization results in a reduced, rather than increased, value of ρ_c , and the polytropic index is not substantially altered.

Thus the asymptotic p-mode frequency separations are well reproduced by the WIMP models, and we have predicted a value ΔP for the separation of high-order g modes of low degree substantially lower than the separations predicted by mixed solar models. Unambiguous g-mode measurements will therefore provide an immediate test to distinguish between the models.

We thank J. Christensen-Dalsgaard for providing eigenfrequencies of his solar model 1 (ref. 23) which we used for Table 1. J.F. and D.O.G. thank the Cambridge Society of Bombay and the Tata Institute of Fundamental Research for hospitality, and J.F. thanks the US NSF for a passage to India. After submitting this letter for publication, we heard that analogous results had been obtained independently by Christensen-Dalsgaard, Däppen and Gilliland from numerical computations of eigenfrequencies of the improved 5-GeV WIMP model. Their results, which confirm our prediction of the effect of WIMPs on the p-mode separations, are reported in the accompanying letter³⁴. This is Lick Observatory Contribution 427.

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Weakly interacting massive particles, solar neutrinos, and solar oscillations

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Weakly interacting, massive particles (WIMPs) have recently been proposed as a solution to the solar neutrino problem^{1–3}. Whereas standard solar models consistently predict a detection rate of (high-energy) neutrinos 3 times higher than that observed in the Davis experiment^{4–6}, the presence of hypothetical massive particles in the solar centre would resolve this discrepancy. Models which incorporate a relative number of 10^{-11} WIMPs with appropriate scattering cross-section would reduce the predicted neutrino detections by ^{37}Cl to the observed value, without significant changes in the solar structure outside the central region^{1–3}. We have subjected these models to an observational test of p-mode oscillation frequencies by computing frequency differences of low-degree, high-order oscillation frequencies. Although standard solar models also pass this test, WIMP models provide a better fit.

The WIMPs, assumed to be thermalized and on Kepler orbits around the solar centre^{1–3}, act through elastic scatterings with the (mainly) hydrogen and helium nuclei, and thus provide an efficient alternative to radiative heat transport. The result is a nearly flat temperature distribution in the central zone and a lower central temperature, which leads to the smaller production rate of high-energy neutrinos. Note that the low-energy neutrino flux (to be measured in the gallium experiment⁶) would not be affected, since it results from the principal energy-generating nuclear reactions, the rates of which are determined by the solar luminosity (at least when solar variations over timescales shorter than the Kelvin–Helmholtz time of 10^7 yr are excluded). The overall effect of models with WIMPs is therefore similar to those with ‘mixing’. Mixed models, however, appear to contradict observed p-mode oscillation frequency-splittings (see below and ref. 8); it is a natural step to subject the WIMP models to the same observational test.

To understand the diagnostic potential of solar oscillation frequencies, asymptotic expressions are very useful, although for the present analysis we have relied on more precise numerical results for the oscillation eigenfrequencies. For p modes of low angular degree l and high radial order n , the eigenfrequencies

are, in the first two asymptotic orders⁸⁻¹⁰

$$\nu_{l,n} = (n + l/2 + \varepsilon^{(p)})\Delta\nu - \frac{[l(l+1) + B]A}{n + l/2 + \varepsilon^{(p)}} \quad (1)$$

Here, $\varepsilon^{(p)}$ is a phase constant of order 1 depending on the physical conditions at the upper and lower turning points of the mode, and B is also a constant of order 1, expressed by a rather complicated integral of the solar model¹¹. In addition,

$$\Delta\nu = \left(2 \int_0^R c^{-1} dr \right)^{-1} \quad (2)$$

where c is the speed of sound, R is the solar radius and

$$A = \frac{1}{2\pi^2} \left[\frac{c(R)}{R} - \int_0^R \frac{dc}{dr} \frac{dr}{r} \right] \quad (3)$$

Because c is small at the surface and large at the centre, $\Delta\nu$ mainly measures the physical conditions at the surface, whereas A is more sensitive to the physical conditions at the centre. A may be determined by calculating frequency differences of the type

$$\delta\nu_{n,l} = \nu_{n,l} - \nu_{n-1,l+2} \quad (4)$$

As is apparent from equation (1), the leading asymptotic term is cancelled in the subtraction and one gets a direct measure of A . For the present study we have adopted a slight reformulation of equation (1)^{10,12-14}, obtained by expanding it about a given $n = n_0$, and taking $x \equiv n + l/2 - n_0$:

$$\nu_{n,l} = \nu_0 - l(l+1)D_0 + [\Delta\nu_0 + l(l+1)d_0]x + O(x^2) \quad (5)$$

D_0 is proportional to A and is therefore suitable as a probe of the solar centre. D_0 is related to the averaged splittings $\delta\nu_{n,l}$ by

$$D_0 \sim \frac{1}{4l+6} \langle \delta\nu_{n,l} \rangle_{n_0} \quad (6)$$

the equation holding only approximately due to the neglect of higher-order terms.

To minimize systematic errors that can arise from using different stellar codes, we have computed D_0 for a standard and WIMP model (numbers 1 and 3 in Table 1 of ref. 3); for comparisons we have also computed this quantity for another standard solar model, Model 1 of Christensen-Dalsgaard^{15,16}. Since $\sim 5\%$ of $\Delta\nu$ comes from the atmosphere⁸, we have attached the atmosphere of Model 1 to the photosphere of the other two models. This procedure is certainly justified by the nearly identical outer structure of all three models and by the fact that any remaining inconsistency will have little influence on the 'central' quantity D_0 . We have satisfied ourselves that all the models are internally consistent by verifying the sound speed in the bulk of the convection zone (which has to be practically the same for all models¹⁰), and the ratios $\delta\nu_{n,1}/\delta\nu_{n,0}$, which, according to equation (6), must be model-independent and equal to 5/3 for high-order p modes. After testing for internal consistency we have computed a set of p modes using an adiabatic pulsation code^{15,16}. We chose the set of modes with $l=0, 1, 2, 3$ and $n=17, 18, \dots, 27$ in all combinations. The frequencies of these 44 modes were then fitted to the polynomial in equation (5), and D_0 was computed. This procedure is different from calculations of the integrals in equations (2) and (3), because the D_0 computed from frequency-fitting of a finite set of modes differs from its asymptotic values. By performing the same fitting procedure for the analogous selection of observed modes, we can make a more reliable test of the models against solar observations than by computing the asymptotic integrals in equations (2) and (3). From the observed frequencies¹⁷⁻¹⁹ (where the same mode was available from more than one observer, equally weighted averages of the frequencies have been used), we have obtained $D_0 = 1.471 \mu\text{Hz}$. The results from our three models are listed in

Table 1 Fitting parameters for four solar models

Model	D_0 (μHz)	P_0 (min)
Standard ³	1.662	36.80
WIMP model 3 ³	1.466	32.56
Model 1 ¹⁰ (standard)	1.643	35.38
Mixed model ⁷	2.065	56.85

Table 1; those for the mixed model ('strongly mixed' with the correct neutrino prediction) are from ref. 9.

In addition, we have computed low-degree, high-order g-mode frequencies and fitted them to the appropriate asymptotic expression (see, for example, ref. 9)

$$\tilde{\nu}_{n,l} = P_0 \frac{n + \varepsilon_l^{(g)}}{\sqrt{l(l+1)}} \quad (7)$$

P_0 , the fundamental g-mode period spacing, is shown in Table 1 for the four models. It has been computed by using all g modes of degree $l=1, 2$ with periods in the range 390–650 min. Because g-mode observations have not yet been satisfactorily confirmed (one of the latest reports is ref. 20), these cannot be used to discriminate among models; however, Dalache and Scherrer²¹ indicated an observed P_0 of 38.6 min. Because g modes have their dominant amplitudes in the central regions of the Sun, they are more strongly affected by changes induced by WIMPs than are the p modes. Identification and confirmation of g modes would thus allow a sensitive test distinguishing between standard and WIMP models.

As noted above, mixed models are not consistent with p-mode observations, in contrast to the agreement obtained for standard and WIMP models. To explain the different D_0 of the three models, consider the asymptotic expression equation (3). Quantitative results based on the complete solar models of Gilliland *et al.*³ can be found elsewhere (W.D., R.L.G. and J.C.-D., in preparation); here we note only that the surface term $c(R)/R$ is small, and that the overall tendency of c is to decrease with increasing r ; this causes the integral to be negative and thus A to be positive. The contribution of the central region is understood by noting that c is proportional to $(T/\mu)^{1/2}$, where μ is the mean molecular mass. In a standard model, T decreases away from the centre, but so does μ because of nuclear evolution. The net results is^{8,10} a fairly flat sound speed as a function of r : there are increasing and decreasing contributions which partially compensate one another in the central part of the integral in equation (3). In a mixed model, T is again decreasing, but μ is flatter because of the homogenization. Here, the net result is a smaller influence of the increasing contribution, causing a smaller compensation and therefore a larger A and D_0 . Finally, in a WIMP model, T is virtually flat, but μ is again decreasing due to nuclear evolution. Therefore, c increases at first strongly in the WIMP region which makes the compensation in the central part more important and thus leads to a smaller A and D_0 .

Observations of solar five-minute frequencies have a relative precision as large as 10^{-4} (ref. 22), which corresponds to $\sim 0.3 \mu\text{Hz}$. A discussion of the propagation of observational errors into D_0 is difficult; however, from the discrepancy between the observed frequencies¹⁷⁻¹⁹ we have estimated a standard deviation of D_0 of $\pm 0.12 \mu\text{Hz}$; therefore, the discrepancy between the observed D_0 and that of the standard models may not be significant. The D_0 of the WIMP model, however, agrees almost exactly with the observed D_0 . Although this may be a coincidence, WIMP models do at least move D_0 in the right direction. This is in contrast to the mixed models mentioned above, for which D_0 is slightly greater than $2 \mu\text{Hz}$ if the mixing is assumed to be strong enough to match the observed ^{37}Cl neutrino flux¹⁰. Mixed models therefore seem to be ruled out

by observation. Solar models with WIMPs are thus the only (astrophysical) solution to the solar neutrino problem that has stood the test of helioseismology.

We thank Peter Gilman for a careful reading of the manuscript and helpful comments, and Tim Brown for stimulating discussions. Having done our calculations, we became aware of the work by Faulkner *et al.*²³, in which they independently came to the same conclusions by evaluating the asymptotic integral, equation (3); this complementary study is a valuable confirmation of our results. We thank these authors for sending their paper and for delaying publication, so that the two approaches could appear together. The National Center for Atmospheric Research (NCAR) is sponsored by NSF.

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Global atmospheric concentrations and source strength of ethane

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Ethane (C_2H_6), like other gaseous hydrocarbons, contributes significantly to the chemistry of the lower atmosphere, chiefly through reactions with the OH (hydroxyl) radical, an important tropospheric oxidizing agent. We present here a study of the variation in C_2H_6 concentration between northern and southern latitudes over 3 years, together with a new estimate of its source strength. Ethane concentrations vary from 0.07 to 3 p.p.b.v. (parts per 10^9 by volume) in air samples collected in remote surface locations in the Pacific (latitude $71^\circ N$ – $47^\circ S$) in all four seasons between September 1984 and June 1985. Earlier remote measurements have generally shown concentrations in the 0.3–3.0 p.p.b.v. range, but coverage over a wide latitude range in the Southern Hemisphere has been limited to single trips^{1–5}. The variations are consistent with southerly transport from sources located chiefly in the Northern Hemisphere, further modified by seasonal variations in the strength of the reaction of C_2H_6 with OH radicals. These global data can be combined with concurrent data for CH_4 (refs 6, 7) and the laboratory reaction rates⁸ of each with OH to provide an estimate of 3 months as the average atmospheric lifetime for C_2H_6 and 13 ± 3 Mtons for its annual atmospheric release.

Our samples were taken in 2-l stainless steel canisters, evacuated in the laboratory and opened to ambient air in remote surface locations (Alaska, North American west coast, Pacific islands)^{6,9,10}. After return to the laboratory, the analysis of these air samples was carried out by gas chromatography with flame ionization detection. The trace components volatile at $-20^\circ C$ were cryogenically trapped from 1 l STP of air, and then analysed on a 3-foot Spherocarb column programmed from -10 to $350^\circ C$, providing a sensitivity of 0.05 p.p.b.v. for C_2H_6 .

The C_2H_6 concentrations measured in our surface air samples varied in the north temperate zone from ~ 0.8 p.p.b.v. in the summer to as much as 3 p.p.b.v. in the winter, and from 0.07 p.p.b.v. (summer) to 0.4 p.p.b.v. (winter) in the south temperate zone. The data for each season are shown in Fig. 1. The Southern Hemisphere concentrations show very little variation with latitude in any given time period. The Northern Hemisphere concentrations are always higher, with especially strong concentration gradients in the northern tropics during December

to March. The major atmospheric removal process for C_2H_6 is the reaction



with OH radicals created by the reaction with H_2O vapour of $O(^1D)$ atoms released by the solar ultraviolet photolysis of tropospheric ozone. About 70–80% of the global OH reactions with C_2H_6 occur in the tropics, with only a weak sink in the temperate zones during winter¹¹. The average concentrations in the zone from 30 to $75^\circ N$ and in the Southern Hemisphere below $10^\circ S$ are given with their 1σ standard deviations in Table 1. The concentrations of CH_4 and of several organochlorine compounds have been analysed on smaller aliquots of the same samples^{6,9,10}.

We have also measured the concentrations of C_2H_6 in Alaskan air samples periodically since March 1983, and have observed a regular seasonal cycle, as shown in Fig. 2. Because the data show no significant differences between samples collected at sites on the north side of the Brooks range (solid symbols) and those from mid-Alaska (open symbols), we conclude that the observed concentrations are representative not of C_2H_6 emitted from local sources such as the Alaskan oil fields, but rather of general levels for northern latitudes. We have fitted these Alaskan data by least squares to a seasonally dependent sine curve, shown as the dashed line in Fig. 2. This empirical fit shows variations in concentration by a factor of 3, and reaches its minimum ~ 6 weeks after the maximum in solar ultraviolet intensity. The seasonal dependence of C_2H_6 concentrations calculated by Isaksen *et al.*¹² from a two-dimensional meridional atmospheric model is shown as the solid line in Fig. 2. The agreement is reasonably good, despite the discrepancy in the time lags introduced because some of the parameters in the atmospheric model were recalculated only quarterly.

The annual average concentration of C_2H_6 throughout the Southern Hemisphere was calculated from the four seasonal

Table 1 Average concentrations of C_2H_6 in remote tropospheric air samples

Collection period	30–75° N		10–50° S	
	No. of samples	Concentration (p.p.b.v.)	No. of samples	Concentration (p.p.b.v.)
September 1984	15	0.86 ± 0.21	8	0.35 ± 0.11
December 1984	20	2.31 ± 0.35	10	0.30 ± 0.09
March 1985	18	2.20 ± 0.39	14	0.14 ± 0.07
June 1985	14	0.93 ± 0.12	13	0.28 ± 0.04

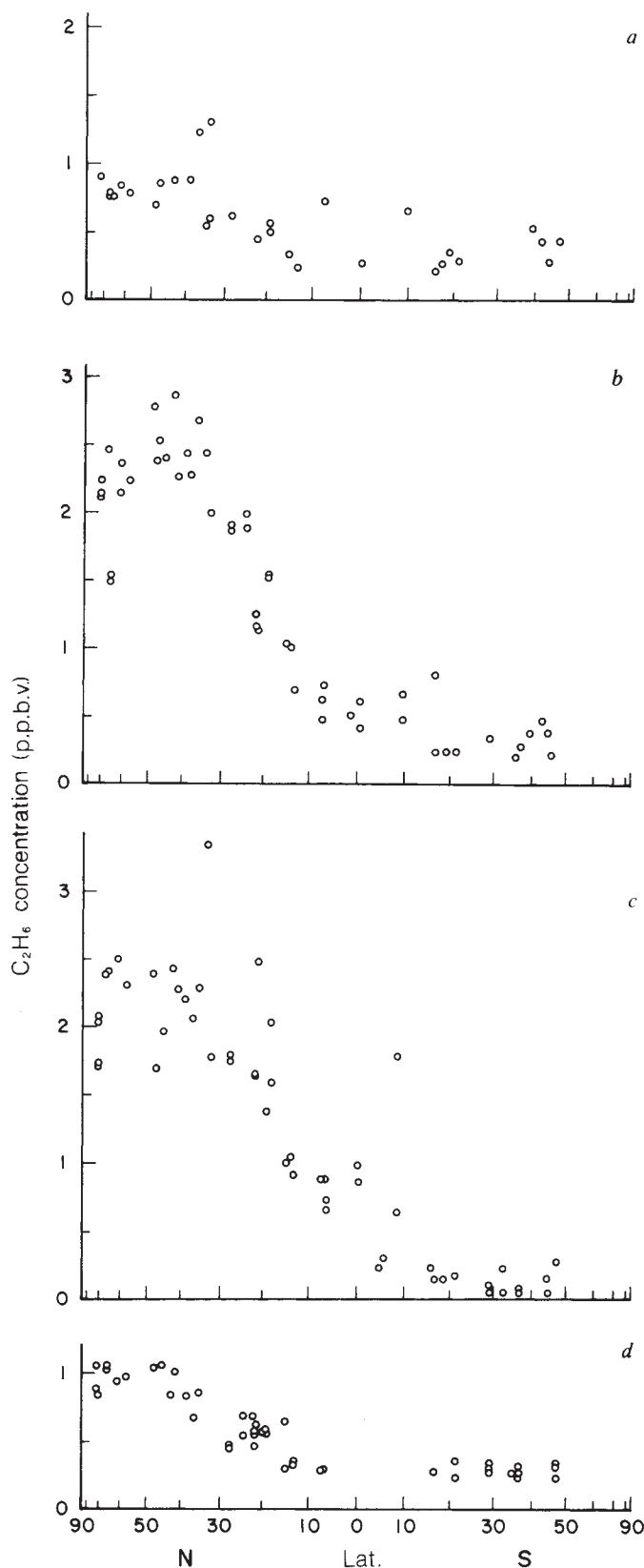


Fig. 1 Measured concentrations of C_2H_6 in p.p.b.v. for four different time periods over the latitude range $71^\circ N$ – $47^\circ S$. *a*, September 1984; *b*, December 1984; *c*, March 1985; *d*, June 1985.

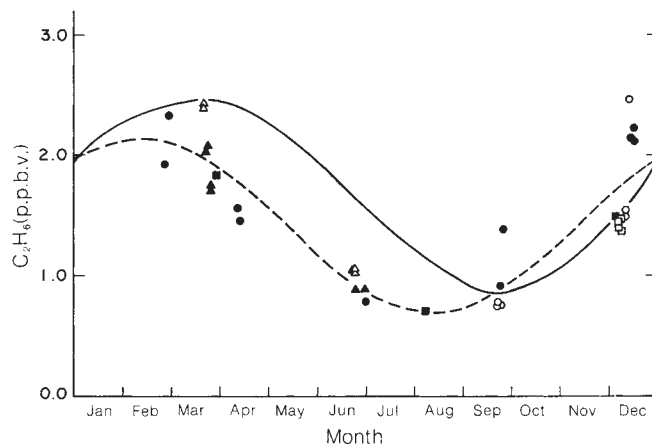


Fig. 2 Measured concentrations of C_2H_6 in p.p.b.v. from Alaska during 1983–85. Solid symbols, north of $70^\circ N$ (■, 1983; ●, 1984; ▲, 1985); open symbols, 65 – $68^\circ N$ (□, 1983; ○, 1984; △, 1985). Dashed line, least-squares fit to sine curve; solid line, atmospheric model of ref. 12.

distributions to be 0.3 p.p.b.v. The annual average concentration for latitudes north of $30^\circ N$ was 1.6 p.p.b.v., while that for the band between the Equator and $30^\circ N$ was 0.8 p.p.b.v. The worldwide concentration of CH_4 measured in the same canisters during these four collection periods was increasing steadily, with a mean of 1,640 p.p.b.v., corresponding to 4,600 Mtons CH_4 in the atmosphere⁷. The atmospheric lifetime of CH_4 cannot be calculated accurately from concentration measurements because of uncertainties in its source strength. However, it can be estimated indirectly by comparison with that of CH_3CCl_3 , the sources of which are better known, through their respective rates of reaction with OH radicals, the controlling process in the atmosphere for each. The atmospheric lifetime of CH_4 has been estimated as 10 yr by comparison with a lifetime of 6.9 yr for CH_3CCl_3 (refs 9, 10), requiring an annual CH_4 emission of ~450–500 Mtons. The rate constant for reaction (1) at 280 K, a rough median temperature for tropospheric OH reactions with hydrocarbons, is 42 times faster than the corresponding reaction with CH_4 (ref. 8), implying an average atmospheric lifetime for C_2H_6 of 3 months. This lifetime is sufficiently short that the survival time before destruction is very much dependent on the time and location of its release.

Assuming that reactions with OH radicals are the dominant removal processes for CH_4 and C_2H_6 , the annual release of C_2H_6 to the atmosphere can be approximately calculated from the ratio of average worldwide concentrations (1,640/0.75), the ratio of reaction rate constants with OH and the molecular masses. Because the concentrations of C_2H_6 are so variable with latitude and season, we have carried out this calculation more accurately by assessing the relative losses in each latitude band, weighted according to the importance in each band of removal of hydrocarbons by OH. With a large fraction of the OH reactions occurring in the tropics, the annual emission total for C_2H_6 is calculated from the band ratios to CH_4 as 13 Mtons, with an estimated accuracy of ± 3 Mtons. The oxidation of C_2H_6 by OH is the major route leading to the formation of peroxyacetyl nitrate (PAN) in the remote atmosphere¹³, and the latitudinal dependence of this source can be calculated from the C_2H_6 data.

In the March series, two air samples from the windward side of the island of Guadalcanal ($9^\circ S$) exhibited very much higher C_2H_6 concentrations than for latitudes on either side. All five samples from Guadalcanal, in three separate collection periods, contained substantially more C_2H_2 , C_2H_4 and C_3H_8 (but not CH_4) than was found in other samples in the Southern Hemisphere. For example, in 1-l STP aliquots from almost all of our other Southern Hemisphere canisters in March 1985, both unsaturated compounds were below our 0.05 p.p.b.v. detection limit,

consistent with the expectation of very rapid removal by reaction with OH during the summer. We do not know whether the origin near Guadalcanal for these C_2 - C_3 hydrocarbons is biological, petroleum-related or from some other source, and plan to investigate these observations more fully in the future. Table 1 includes only Southern Hemisphere data from 10 to 50° S, to avoid the uncertainty about the significance of these data from Guadalcanal.

A previous estimate of C_2H_6 concentrations in remote tropospheric locations gave ranges of 1.0–1.5 p.p.b.v. in the Northern Hemisphere and 0.3–0.6 p.p.b.v. in the Southern Hemisphere⁵, although values outside these ranges were also reported^{1–4}. The Southern Hemisphere data in particular were based on short-term studies, none of which covered the late Austral summer period in which we observed the lowest concentrations. Measurements of C_2H_6 during 1979–81 at Point Arena, California (39° N), showed no seasonal cycle in concentrations, with average values >2 p.p.b.v. throughout most of the spring and summer². These summertime concentrations are considerably higher than those shown in Fig. 1 for such latitudes, and suggest that the apparent absence of a seasonal cycle may have been caused by C_2H_6 additions from local sources.

The observation of a monotonic increase in atmospheric CH_4 concentrations^{6,7,14–16} has led to the postulate that the average worldwide OH concentration has been gradually decreasing over the past 10 yr, and probably over the past 40 yr (refs 7, 16–20). The C_2H_6 concentrations that we measured in the north temperate zone are often higher than those given by Rudolph and Ehhalt for the same latitude band a few years earlier^{1,3,4}.

However, the variations in C_2H_6 concentration with season and latitude are sufficiently large that we do not believe that the larger Northern Hemisphere values in our experiments necessarily represent an upward trend in these values during the 1980s. The precision of our measurements for C_2H_6 is, of course, not sufficient to detect changes of the order of 1% per yr, the rate of increase found for tropospheric CH_4 .

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Helium: a new tracer in Antarctic oceanography

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The abyssal characteristics of the world oceans are strongly influenced by the northward propagation of Antarctic Bottom Water (AABW). An important source of AABW is Weddell Sea Bottom Water (WSBW), which is formed, in part, on the continental slope of the southern Weddell Sea^{1–3}. The formation of WSBW on the continental slope is related to the floating ice shelves of the southern Weddell Sea (Filchner/Ronne Ice Shelves). Western Shelf Water (WSW) is modified under the ice shelves by cooling and admixture of melt water to form Ice Shelf Water (ISW), and a substantial part of the ISW flows over the sill that separates the Filchner Depression from the Weddell Sea and participates in the formation of WSBW^{4,5}. The data reported here demonstrate that the water/ice interaction leads to a strong 4He -supersaturation of the ISW due to dissolution of air entrapped in the ice-shelf melt water. The 4He -supersaturation of the ISW can be used as a tracer of this water mass and also influences the 4He balance of the WSBW.

Figure 2a shows a 4He concentration profile from a station near the Filchner Ice Shelf (Fig. 1), which was occupied during the third Antarctic expedition of the German polar research vessel *Polarstern* in February 1985. The corresponding temperature and salinity profiles are plotted in Fig. 2b, and all the data are listed in Table 1. Helium samples were collected from 12-l Niskin bottles and stored using pinched-off copper tubes. He isotope concentrations were measured at Heidelberg with a special $^3He/^4He$ mass spectrometer (MM3000, VG Instruments, Winsford), an instrument similar to that described by Clarke *et al.*⁶. The data were calibrated to air standards⁷. To avoid interferences, neon was separated from helium before admitting the

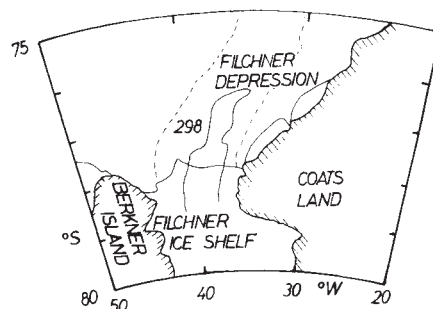


Fig. 1 Geographical position of station 298.

gas to the mass spectrometer. Errors arising from inclusion of air in the water samples during collection are estimated to be about $\pm 1\%$ on the basis of 4He measurement on duplicate samples. The overall reproducibility is estimated to be about $\pm 1\%$ (ref. 8).

The temperature profile plotted in Fig. 2b shows surface values of $\sim -1.2^\circ C$, decreasing to $\sim -1.9^\circ C$ near 300 m depth. Below this depth, ISW is found, which is characterized by temperatures below the surface-water freezing point of $\sim -1.9^\circ C$. The core of the ISW, marked by a temperature minimum of $-2.19^\circ C$, is centred at ~ 425 m depth. Salinities increase steadily from $\sim 34.05\%$ at the surface to 34.60% in the ISW core, without much reflection of the water-mass structure.

The 4He concentrations decrease from a value of $\sim 4.4 \times 10^{-8} \text{ cm}^3 \text{ STP g}^{-1} \text{ H}_2\text{O}$ at the surface to $\sim 4.20 \times 10^{-8} \text{ cm}^3 \text{ STP g}^{-1} \text{ H}_2\text{O}$ between 50 and 150 m (see Fig. 2a). Below this depth they increase again, reaching a maximum value of $\sim 4.8 \times 10^{-8} \text{ cm}^3 \text{ STP g}^{-1} \text{ H}_2\text{O}$ in the core of the ISW, and then decrease towards a value of $\sim 4.65 \times 10^{-8} \text{ cm}^3 \text{ STP g}^{-1} \text{ H}_2\text{O}$. Compared with normal sea water, the 4He concentrations of the ISW are exceptionally high. Excess over an air/sea solubility equilibrium (given as $\Delta^4He = ((^4He_{\text{sample}}/^4He_{\text{sat}}) - 1) \times 100\%$, where $^4He_{\text{sat}}$ is the 4He saturation concentration after Weiss⁹) ranges from ~ 5 to 19% , with a maximum value in the core of the ISW (see

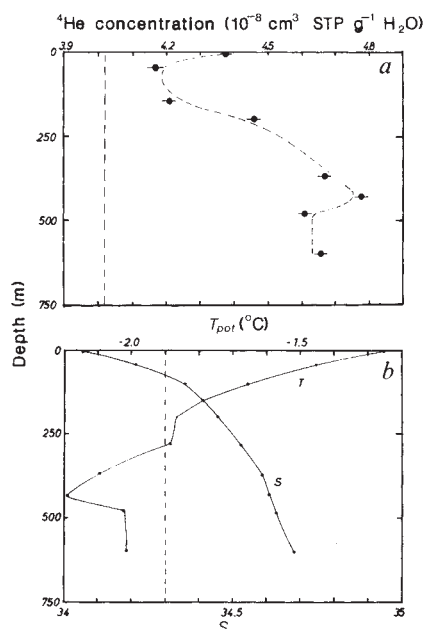


Fig. 2 *a*, ^4He concentration profile from station 298. The vertical dashed line shows the equilibrium solubility of ^4He (ref. 9). *b*, Potential temperature and salinity profiles from station 298. The dashed line indicates the surface-water freezing point.

Table 1). ^4He supersaturation in ocean surface water is usually only $\sim 5\%$ (ref. 10). Thus, the ^4He supersaturation of the ISW core exceeds that normally found in sea water by $>10\%$.

The high ^4He concentrations of the ISW can be assumed to be introduced during the formation of this water mass: the ice at the underside of the ice shelves which is melted to form ISW contains air that has been entrapped during ice formation, which is present in small bubbles under high pressure. During ice melting it becomes dissolved in the melt water, and the pure melt water is therefore extremely supersaturated in gas content. The excess relative to a solubility equilibrium caused by such addition of air can be calculated from

$$\Delta' = \left(\frac{((\alpha \times C_{\text{air}} + f \times C_{\text{air}}) / (\alpha \times C_{\text{air}})) - 1}{f / (\alpha \times 100)} \right) \times 100\% \quad (1)$$

$$= (f / (\alpha \times 100))\%$$

where Δ' is the excess above solubility equilibrium with air, C_{air} the gas concentration in air, α the Ostwald coefficient, and f the volume fraction of air added.

Thus, for a given fraction of air added, the excess gas concentration above solubility equilibrium increases with decreasing solubility of the gas. This means that high excesses can be expected, especially for the light noble gases helium and neon which both have low solubilities. For ^4He , with an Ostwald coefficient of 9.4×10^{-3} in fresh water at 0°C (ref. 14), equation (1) indicates that the melt water will be supersaturated by a factor of 14 (for an assumed ice density of 0.91 g cm^{-3} (ref. 11)), if as a first-order approximation, the air content of the ice is assumed to be $0.12 \text{ cm}^3 \text{ STP g}^{-1}$, as found for a core taken at Byrd station¹². Thus, even small contributions of melt water to the ISW produce a detectable ^4He anomaly in this water mass. With a ^4He supersaturation in the melt water of $\sim 1,400\%$ and a ^4He measuring precision of about $\pm 1\%$, it follows that $\sim 0.07\%$ of melt water in the ISW should be detectable.

The source of the excess ^4He can be verified by means of $^3\text{He}/^4\text{He}$ ratio measurements. The $^3\text{He}/^4\text{He}$ ratio of air is $\sim 1.4 \times 10^{-6}$ (ref. 6). If dissolution of air bubbles is indeed the source of the ^4He excess, the $^3\text{He}/^4\text{He}$ data points should fall on a straight line with a slope of 1.4×10^{-6} in a plot of ^3He versus ^4He concentration. Figure 3 shows that this is the case for the

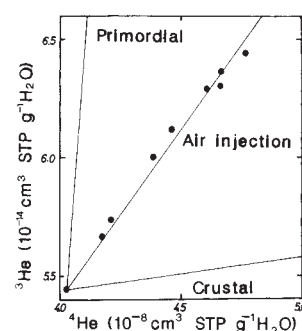


Fig. 3 ^3He versus ^4He plot for station 298. The slopes for injection of helium derived from the air, the Earth's mantle (primordial) and the Earth's crust (crustal) are indicated.

Table 1 Hydrographic and helium isotope data from station 298

Pressure (dbar)	T_{pot}^* ($^{\circ}\text{C}$)	S (‰)	^4He conc. ($10^{-8} \text{ cm}^3 \text{ STP g}^{-1}$)	$\Delta^4\text{He}$ (%)	$\delta^3\text{He}^\dagger$ (‰)
3	-1.250	34.053	4.38	9.0	-0.92‡
42	-1.453	34.229	4.17	3.7	-1.75
149	-1.786	34.430	4.21	4.7	-1.51
199	-1.865	34.463	4.46	11.0	-0.90
369	-2.094	34.593	4.67	16.2	-1.63
429	-2.192	34.611	4.77	18.7	-2.49
480	-2.024	34.628	4.61	14.7	-1.42
599	-2.017	34.681	4.66	15.9	-2.27

* CTD data.

† $\delta^3\text{He} = (R_{\text{sample}} - R_{\text{air}}) / R_{\text{air}} \times 100\%$, where R_{sample} is the $^3\text{He}/^4\text{He}$ ratio of the sample and R_{air} the $^3\text{He}/^4\text{He}$ ratio of air.

‡ Measurement precision is $\pm 0.15\%$.

helium data of station 298. Thus, at least the major part of the ^4He excess of the ISW is due to the presence of dissolved air. An addition of small fractions of radiogenic or primordial helium cannot be excluded on the basis of the present data; however, they could be excluded by additional neon measurements. Neon has a low solubility in sea water, but no sources in the ocean interior have been identified. Further studies should therefore include neon measurements.

The ^4He excess of the ISW can be used to estimate the fraction of melt water contained in the ISW. Assuming that the helium contents of waters collected between 50 and 150 m at station 298 are representative of the water mass which is first modified to WSW and then to ISW, the ISW shows a ^4He excess of $\sim 14\%$ relative to this initial water type. If this excess is ascribed to addition of air during ice melting, a fraction of 1.0% of melt water is obtained for the ISW core at station 298.

This estimate of melt water admixture to the ISW can be compared with one based on a salinity balance. Given the salinity difference between WSW (34.75‰) and ISW (34.60‰) of 0.15‰ (refs 4, 5), a fraction of 0.43% of melt water is necessary to satisfy the salinity balance. This is lower than the fraction required by the ^4He balance (1.0%, see above); however, the salinity effect may be masked by a salinity increase caused by the brine release which precedes melt water addition under the ice shelf⁴. Furthermore, Weiss *et al.*³ derive from stable isotope measurements that WSW, before its alteration to ISW, already has 0.32% of melt water admixed. These two melt water components; that is, 0.32% in the WSW and an additional 0.43% from under the ice shelf, add up to 0.75%, which is reasonably close to the value calculated from the ^4He excess. An additional difference may be caused by freezing of water at the underside of the ice shelves. Such freezing would increase the salinity of the ISW (by brine release) but would have only a minor influence on the helium and ^{18}O concentrations of this water mass.

The sensitivity of noble gases as tracers for ice-shelf melt water can be compared with that of stable isotopes. (For details about the application of stable isotopes in studies of oceanic processes see ref. 13.) Weiss *et al.*³ find that the ISW is exceptionally depleted in $\delta^{18}\text{O}$ due to admixture of isotopically extremely light melt water from the Filchner Ice Shelf. This fact enables the application of stable isotopes as a tracer of ISW in the formation process of the WSBW. Weiss *et al.* estimated a $\delta^{18}\text{O}$ value for the melt water of about -50% . Their measurement of stable isotopes in ISW yielded a $\delta^{18}\text{O}$ value of $\sim -0.6\%$; thus, the difference in $\delta^{18}\text{O}$ between melt water and ISW is $\sim -50\%$. With a measurement precision of $\sim 0.02\%$, a fraction of 0.04% of melt water in the ISW can be detected. The corresponding detection limit for the ^4He -based estimate is given above as 0.07% . It should be a straightforward matter, however, to halve this limit; for example, by collecting duplicate samples and by closely monitoring mass spectrometer linearity. The resolution based on helium measurements will then be the same as that from stable isotope measurements.

ISW participates in the formation of WSBW, and thus the helium content of WSBW is influenced by the ice shelf melt water. First measurements of WSBW samples (P.S., unpublished data) indicate a ^4He excess of $\sim 1\text{--}2\%$ due to addition of melt water to the WSBW. The ice shelves therefore seem to have a considerable influence on the gas balance of the abyssal waters

found in the Weddell Sea. A quantitative treatment of this effect requires further detailed measurements.

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Evidence for the existence and development of visual inhibition in humans

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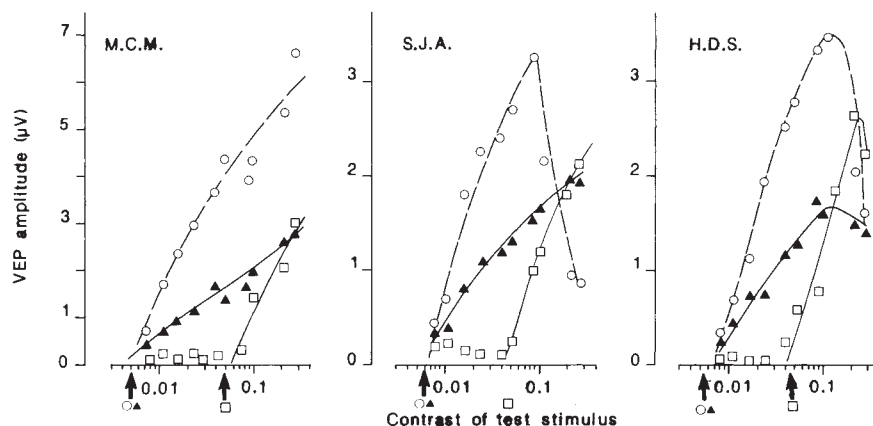
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Neural inhibition forms a major mechanism by which the nervous system refines and elaborates its input¹. Several recent experiments have demonstrated the existence of inhibition between orientation-selective cells of the primary visual cortex of the cat^{2–7} and although the precise function of this inhibition is uncertain, there is evidence that it enhances orientation tuning⁷ and that it is involved in pattern recognition⁸. Here we report a series of experiments which, on the basis of evoked potential responses to oriented stimuli, suggest that similar processes may exist in humans. Recordings from young infants further suggest that the machinery which mediates orientation-specific interactions may not be functional at birth, but develops only after 6–8 months.

The stimuli used in these studies were sinusoidal gratings: a test of variable contrast and a high-contrast (20%) mask of slightly different spatial frequency, oriented either orthogonal or parallel to the test. Both test and mask gratings were phase reversed at rates from 3 to 7 Hz. Visually evoked potentials (VEPs) were appropriately filtered, amplified and averaged (by computer) in synchrony with the test phase reversal. Averaging at this frequency accumulated the potentials generated by the test, but not those generated directly by the mask (which was phase reversed at a different frequency). Measurements were made on three adults, all of whom had corrected 6/6 vision, and on three infants, all of whom had normal vision (1–2 dioptre hypermetropia), over a period of 8 months.

Some of the results for the adults are presented in Fig. 1. They show the amplitude of the evoked potential as a function of test grating contrast, measured with no mask and with superimposed orthogonal or parallel masks. In the absence of any mask, the VEP modulated at twice the temporal frequency of the test, with very little modulation at the first, third or fourth harmonics. The amplitude of modulation increased monotonically (roughly logarithmically) with contrast up to a saturation point, then began to decrease (except for M.C.M.). Like Campbell and Maffei⁹, we find that extrapolation of contrast

Fig. 1 Amplitude of the second harmonic of VEP modulation as a function of grating contrast. The test was a sinusoidal grating of 1 cycle deg^{-1} measured with no mask ($\circ$) and with masks of 0.8 cycles deg^{-1} orthogonal ($\blacktriangle$) or parallel ($\square$) to the test grating. The temporal frequency of the test was chosen to be just high enough to give a clear second-harmonic response for each observer, with no significant modulation at the higher harmonics (M.C.M., 7.2 Hz; S.J.A., 10 Hz; H.D.S., 7.2 Hz). The mask was modulated at 0.8 times this frequency. The arrows indicate the psychophysical detection thresholds, measured for the three conditions using the method of adjustment (average of five settings). The screen subtended a 30-cm square and had a mean luminance of 40 cd m^{-2} . Evoked potentials were differentially recorded with electrodes on the vertex and 2.5 cm above theinion, with a third electrode on the forehead serving as earth. The average multiplicative factors by which the slope of the contrast response curve was attenuated by the orthogonal mask were 0.38 ± 0.04 for M.C.M., 0.39 for S.J.A. and 0.33 ± 0.03 for H.D.S.



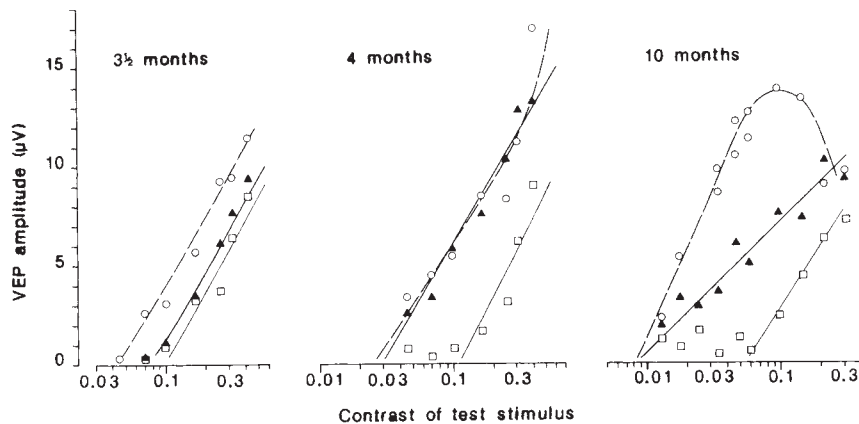


Fig. 2 Representative contrast response curves for one of the infants (T.L.B.) at 3½, 4 and 10 months: ○, no mask; ▲, orthogonal mask; □, parallel mask (see Fig. 1 legend for experimental details). Spatial and temporal frequencies were 0.8 cycles deg^{-1} , 5 Hz at 3½ months, and 1 cycle deg^{-1} , 7.2 Hz for the other curves. The recording electrodes were positioned on the vertex and 1.5 cm above the inion, with the earth electrode on the forehead.

response curves efficiently predicts psychophysical thresholds. Superposition of the orthogonal mask did not affect the form of the VEP (by introducing higher or lower harmonics) but significantly reduced the amplitude of second-harmonic modulation. The reduction was greater at high than at low contrasts, yielding contrast response curves of lower slope. Interestingly, the orthogonal mask had very little effect on threshold, either that measured psychophysically or the VEP estimate. The attenuation produced by the mask is multiplicative (or divisive), reducing the contrast response curve without significantly affecting contrast threshold.

To be sure that the multiplicative attenuation results from an interaction between mechanisms tuned to different orientations, we also measured the effect of a superimposed parallel mask (Fig. 1). This mask also attenuated the VEP amplitudes^{10,11}, but the attenuation shifted the contrast response curve to the right, without lowering its slope. Extrapolation of the curve again predicts the psychophysically measured threshold, which is much higher than that measured either with no mask or with the orthogonal mask^{12,13}. As a further control, we measured VEP response with orthogonal masks of varying spatial frequency. Masks of spatial frequencies differing by more than two octaves from the test had no effect on VEP amplitude. Change of the contrast response curve is specific to masks of different orientations but with similar spatial frequencies to those of the test.

Measurements were made over a wide range of test spatial frequencies (keeping the mask at 0.8 times the frequency of the test). For all test frequencies from 0.5 to 10 cycles deg^{-1} (the range giving reliable contrast response curves), the orthogonal mask reduced the slope of the contrast response curve to a degree comparable to that shown in Fig. 1. The effect was also obtained over a range of temporal frequencies from 5 to 14 Hz.

Contrast response curves (with and without masking gratings) were also measured for three infants over an 8-month time span, to monitor the developmental course of the effect. Figure 2 shows samples of the results for one of the infants (T.L.B.) at 3½ months, 4 months and 10 months. At 3½ months, the three curves are virtually parallel. Those curves for the parallel mask and the orthogonal mask are superimposed, both slightly below the curve for no mask. Two weeks later, the pattern has changed. The curves are still parallel, but that with the orthogonal mask is now coincident with the no-mask curve, both of which are higher than the curve for the parallel mask. At 10 months the pattern is virtually the same as for adults, with the orthogonal noise producing a change in slope and the parallel noise a uniform depression. As the choice of spatial and temporal frequencies could be relevant, for one of the infants (T.L.B.) we measured contrast response curves with a range of spatial and temporal frequencies (0.5–2 cycles deg^{-1} ; 3–7.2 Hz), at various stages. There was no evidence of multiplicative attenuation at any frequency before 6 months of age (although very low temporal

frequencies were not used for technical reasons). We have also repeated the experiments with kittens¹⁴ (where more extensive measurements were possible), and shown that the effect is not present before 40 days, regardless of the spatial or temporal frequency of the stimulus (0.05–0.8 cycles deg^{-1} ; 0.8–7 Hz).

An estimate of the average multiplicative factor by which the orthogonal mask attenuated the VEP amplitude was obtained by plotting the VEP amplitudes measured with the orthogonal mask against those measured with no mask, and calculating the best linear estimate of the relationship (by least-squares fit). The slope of the equation of linear fit provides an estimate of the multiplicative factor of attenuation, with its associated error. Figure 3 plots the multiplication factor for the three infants as a function of age. Up to about 6 months the factor is ~1, indicating that the orthogonal mask had no effect on the slope of the contrast response curves. After this, the multiplication factor begins to decrease rapidly, reaching adult values at about 8 months.

Previous studies have shown that the VEP response to modulated stimuli can be modified by addition of superimposed^{10,11,15,16} or adjacent^{17,18} masks modulated at different temporal frequencies. These results have been explained by rectifying¹⁶ or multiplicative¹⁸ nonlinearities in the neural mechanisms that generate the VEP. Here we extend these studies by showing that the VEP contrast response curves are modified by parallel and orthogonal masks in a different way, implying an orientation-specific interaction: the orthogonal mask lowers the slope of the curve, while the parallel mask does not. Attenuation of contrast response slope, without a significant accompanying change in threshold, is similar to results found in cells in the cat primary visual cortex when a stimulus orthogonal to the cell's preferred orientation is introduced⁴. It is likely that the multiplicative attenuation of single cortical cell response is mediated by inhibitory processes and that γ -aminobutyric acid (GABA) is the transmitter of the inhibition^{5–7}. Recent experiments in our laboratory provide similar evidence that the attenuation of VEPs (in cat) by orthogonal stimuli is also GABA-mediated¹⁹. When bicuculline (a GABA blocker) is applied to the cortex (either topically or iontophoretically), an orthogonal mask does not reduce the slope of the VEP contrast response curve (while under normal conditions it does). It is possible that the effect reported here on human VEPs, which parallels so closely that observed in both single-cell and VEP recordings in cats, also results from inhibitory interactions between neurones of different orientations, and is GABA-mediated.

The results shown in Figs 2 and 3 probably reflect the development of orientational selectivity and of cross-orientation inhibition in humans. For the early measurements, up to 3½ months, the orthogonal mask had a similar effect to the parallel mask: uniform depression of the whole response curve, lowering the (extrapolated) contrast threshold. This would occur if cortical

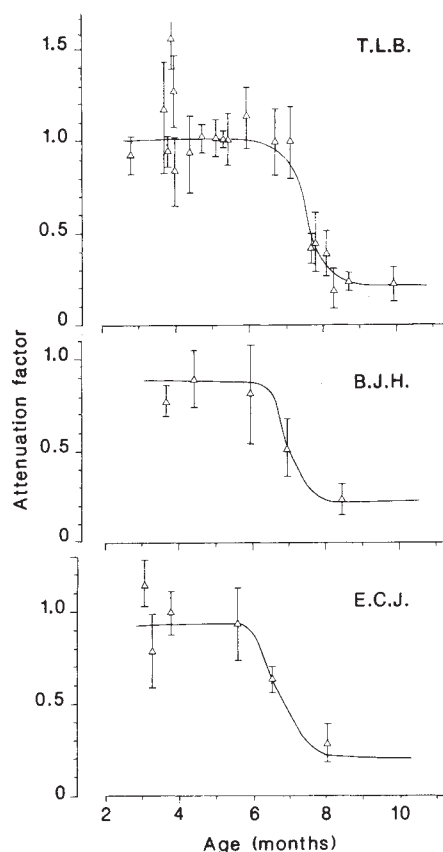


Fig. 3 Effect of orthogonal masks on the slope of the contrast response curve as a function of age for three infants. The index of attenuation is calculated by linear fit of the VEP amplitudes measured with the orthogonal mask with those measured without it. Measurements of all contrasts up to the point of saturation were used for the fit. The correlation between the linear fit and the data was always in the range of 0.75–0.95. The ordinate plots the linear coefficient of the fit, and the bars the associated error. Multiplicative factors around 1 imply no multiplicative attenuation, while 0.25 implies that all VEP amplitudes were reduced to one-quarter of their amplitude. The value of 0.25, found after 8 months, is comparable to that obtained with adults. For T.L.B. the temporal frequency varied between 3 and 7.2 Hz (between sessions) and spatial frequency between 0.2 and 2 cycles deg^{-1} up to the age of 5 months, and held at 7.2 Hz and 1 cycle deg^{-1} thereafter. For the other infants the temporal frequency was 5 Hz up to 5 months, and 7.2 Hz thereafter, with spatial frequency always 1 cycle deg^{-1} . The variation in temporal and spatial frequencies did not affect the multiplicative factor.

neurons of young infants were broadly tuned for orientation (as are kitten cells^{20–22}), causing the orthogonal mask to stimulate the same cells that respond to the test. Later in development, around 4 months, the orthogonal mask has no effect at all, suggesting that at this age the cells have some selectivity for orientation, but that inhibitory mechanisms which change the slope of the curve are not yet in place. These mechanisms do not appear until about 6 months, whereupon they mature rapidly.

There is strong evidence that acuity and contrast sensitivity of infant vision improve steadily with age^{23–29}, with most of the development occurring within the first 6 months³⁰. Fine-resolution tasks such as stereopsis are also adult-like by 6 months of age³⁰. However, it is not until 6 months of age that we find evidence for cross-orientation inhibition. This result is interesting, as several physiological^{31,32} and anatomical³³ studies have shown that in the cat, inhibition develops after most of the functions associated with excitation have matured.

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A comparison of inhibition in orientation and spatial frequency selectivity of cat visual cortex

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Neurons in the visual cortex are highly selective for orientation¹ and spatial frequency^{2,3} of visual stimuli. There is strong neurophysiological evidence that orientation selectivity is enhanced by inhibitory interconnections between columns in the cortex which have different orientation sensitivities^{4–6}, an idea which is supported by experiments using neuropharmacological manipulation^{7,8} or complex visual stimuli⁶. It has also been proposed that selectivity for spatial frequency is mediated in part by a similar mechanism to that for orientation, although evidence for this is based on special use of visual stimuli, which hampers interpretation of the findings^{9,10}. We have therefore examined selectivity for both orientation and spatial frequency using a technique which allows direct inferences about inhibitory processes. Our method uses microiontophoresis of an excitatory amino acid to elevate maintained discharge of single neurons in the visual cortex. We then present visual stimuli both within and outside the range of orientations and spatial frequencies which cause a cell to respond with increased discharge. Our results show that orientations presented on either side of the responsive range usually produce clear

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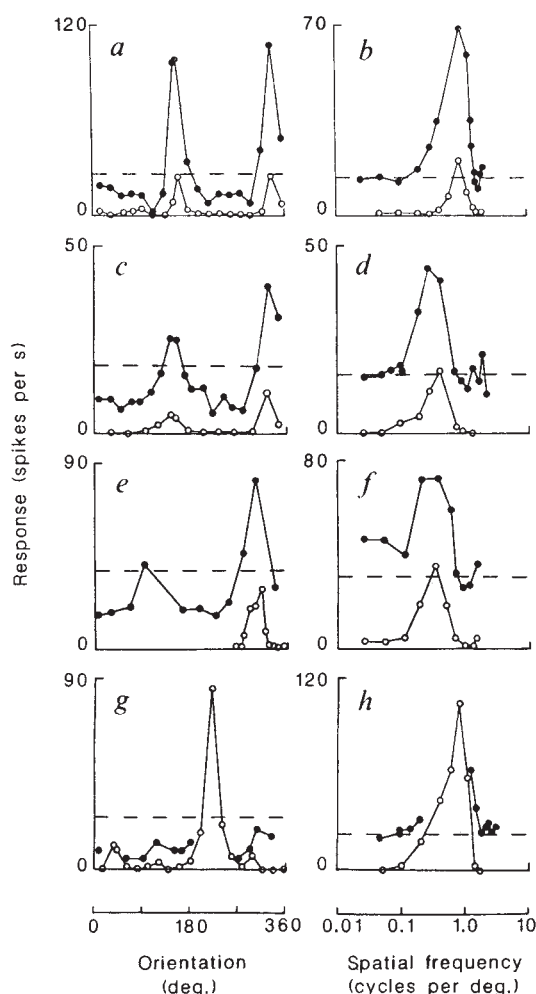


Fig. 1 Responses of four cortical cells to variation in the orientation (*a, c, e, g*) or spatial frequency (*b, d, f, h*) of a stimulus (drifting sinusoidal gratings). Solid lines represent responses before (open symbols) and during (closed symbols) ejection of DL-Hcy. In *g* and *h*, only non-optimal stimuli were tested during drug ejection. Maintained activity, originally close to zero, was raised by DL-Hcy to the levels shown by the dashed lines. Although DL-Hcy increased spontaneous activity and strength of responses, no significant changes were observed in orientation and spatial frequency selectivity at the current levels used for ejection. Note that maintained discharge is suppressed by orientations outside the tuning functions. In contrast, little or no effects are observed with stimuli having spatial frequencies on either side of the responsive range.

suppression of maintained discharge. In marked contrast, spatial frequencies shown to either side of the responsive range have little or no effect on maintained activity. We conclude that there is an intracortical organization of inhibitory connections between cells tuned to different orientations but not different spatial frequencies.

Normal adult cats were prepared for single-unit recording using standard procedures¹¹. Three-barrelled glass micropipettes were used to record from single neurones and to deliver chemical agents. The first barrel contained a tungsten wire with a protruding tip for recording; the second barrel was filled with either DL-homocysteate (DL-Hcy; 0.5 M, pH 7.4) or L-glutamate (L-Glu; 0.2 M, pH 7.4); and the third barrel contained 0.9% NaCl solution, which was used to cancel the current used for ejection of the drugs. The device used for iontophoresis consisted of two sources of constant current.

We searched for cells using manually controlled slits and bars of light of varying dimensions. For each cell recorded, detailed receptive fields were plotted qualitatively and the neurone was

classified as simple or complex (hypercomplex types were not studied)¹². The receptive field for the dominant eye was then aligned in the centre of a large, bright video display. Next, estimates of preferred orientation and spatial frequency were made qualitatively, and then quantitative runs were conducted, first for orientation and then for spatial frequency selectivity, using computer-controlled stimuli (gratings with sinusoidal luminance distribution). Sequences were run in pseudo-randomly interleaved fashion to minimize effects of response variability. Each variable was tested 10–20 times for 2 s per presentation, and blank screens with the same mean luminance as that of the gratings were included in each test sequence to obtain at least two estimates of spontaneous discharge rates.

After a complete set of data had been obtained for each cell, DL-Hcy (–10 to 0 nA) or L-Glu (–30 to 0 nA) was ejected to raise maintained discharge rates and a set of quantitative measurements was made again. A third and final run was made with the drugs retained at around 12 nA. At the end of each penetration, a lesion was made and subsequent histological analysis was performed to confirm that units were located in area 17.

Of the 117 cells recorded from 10 cats, complete data (tests for both orientation and spatial frequency) were obtained from 94 units. Results were collected in three different ways. First, for 10 cells, maintained discharge was elevated by iontophoretic application of L-Glu. Second, for most cells (90) DL-Hcy was used. Compared with L-Glu, this drug provided stronger excitatory effects, obtained with lower ejection currents. Third, 17 neurones exhibited sufficient spontaneous activity for them to be studied without iontophoresis. All three methods yielded similar results, suggesting that the effects observed were not drug-specific or drug-generated.

Figure 1 shows representative results for complex cells (Fig. 1*a, b, e, f*) and simple cells (Fig. 1*c, d, g, h*). Maintained activity, originally close to zero in each case, was raised with DL-Hcy. For these and other cells, simple cells generally required higher ejection currents than complex, reflecting their lower natural spontaneous levels^{13,14}. Note that in each case, the data show that cells are selective for a restricted range of orientations and spatial frequencies. Application of the excitatory amino acid causes increased maintained discharge and a raised excitatory response. The important result for all four cells is that discharge is clearly depressed for orientations outside the excitatory range. In marked contrast, this does not occur in the case of spatial frequency, for which only very weak or no inhibitory side bands are observed. Note that the number of points tested on either side of the excitatory range is relatively large, so it is unlikely that inhibitory frequencies have been missed.

Similar results were observed for nearly all cells tested. To estimate the extent of suppression for all tests conducted, we calculated the difference between maintained discharge and suppressed activity levels for the entire sample. In the case of spatial frequency, we computed separate values for each side of the optimal since, on the basis of a previous report, we anticipated possible differences between high and low frequencies⁹. For these computations, we used orientations and spatial frequencies which had maximal suppressive effects on maintained activity. Results for all cells are shown in Fig. 2, in which findings are compared between spatial frequencies above (*a*) and below (*b*) optimal values. Data for orientation are shown in Fig. 2*c*. Effects are expressed in terms of percentage reduction of maintained discharge; thus, 100% represents complete suppression. The distributions for high and low spatial frequencies are similar but significantly different from that of orientation. For most cells, suppression for orientation tuning side bands is prominent, but this is not generally found for spatial frequency. Note that the inhibitory effects we have observed could be due in part to stimulation of receptive field surrounds as well as central regions.

The marked difference between orientation and spatial

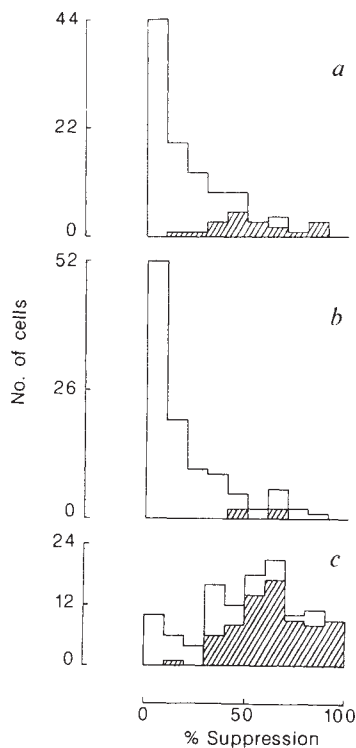


Fig. 2 Histograms illustrating the amount of suppression of maintained discharge levels during variation of spatial frequency above (a) and below (b) the optimal values. Similar data for variation of orientation are shown in c. The overall distributions for high and low spatial frequencies are similar, but significantly different from that of orientation (Wilcoxon signed rank test, $P < 0.001$). The shaded area shows the distribution of cells showing a statistically significant suppressive effect (t -test, $P < 0.01$).

frequency tuning side bands seems to be inconsistent with previous investigations in which maintained activity was raised with visual stimulation (a second grating was used)^{9,10}. In these previous studies, suppressive effects were reported for spatial frequencies outside the responsive range^{9,10}. However, the use of a second visual stimulus is problematic for two reasons. First, the second or conditioning stimulus activates neurones in the visual pathway prior to the visual cortex, and these lower level units could mediate inhibitory interactions. Second, a conditioning grating superimposed on a test grating can cause local variations in contrast. We tested a small sample of cells to determine whether these problems are important. First, we recorded from cortical cells and raised maintained discharge using a second superimposed grating. Suppression was found in all cases but for 9 out of 12 cells, it was clearly dependent on the relative position of the two stimuli. Second, we recorded from cells more peripheral than visual cortex, in the lateral geniculate nucleus. Once again, position-dependent suppressive effects were found for 6 out of 9 cells. We conclude that local variations in the contrast of the compound grating stimulus make it difficult to interpret any observed suppressive effects.

Suppression by orientations outside the excitatory tuning range may play an important role in visual function. By analogy with the retina, where inhibition from the receptive field surround seems to be involved in the analysis of differences in luminance, intracortical inhibition could enhance orientation differences in the environment. Our results suggest that a cortical organization subserving this role is not present for spatial frequency.

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Cytoimmunotherapy for persistent virus infection reveals a unique clearance pattern from the central nervous system

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The mechanism(s) by which infectious or malignant material is cleared by the host has long been an area of intensive study. We have used the murine model of infection with lymphocytic choriomeningitis virus (LCMV) to look at immune clearance during persistent infection. LCMV was selected because the mouse is its natural host, it easily induces acute or persistent infection *in vivo*, and the mechanism by which it is cleared *in vivo* during acute infection is now well understood¹⁻⁸. Clearance, although associated with several antiviral immune effector mechanisms¹, is primarily dependent on the activity of virus-specific cytotoxic T lymphocytes (CTL) restricted by H-2 molecules of the mouse major histocompatibility complex (MHC)⁴⁻⁸. If these cells fail to generate or are depleted, progression from acute to persistent infection occurs^{1,9,10}. Here, using molecular probes, we show that viral nucleic acid sequences, viral proteins and infectious materials can be efficiently and effectively cleared by adoptive transfer of antiviral H-2-restricted lymphocytes bearing the *Lyt 2*⁺ phenotype. Viral materials are cleared from a wide variety of tissues and organs where they normally lodge during persistent infection. Unexpectedly, the mode by which viral materials are removed from the central nervous system (CNS) differed markedly from the mechanism of clearance occurring at other sites. These observations indicate the possible use of adoptive lymphocyte therapy for treatment of persistent infections and suggest that immune clearance of products from the CNS probably occurs by a process distinct from those in other organs.

After injection of virus, infection persists in many organs with virus reaching its highest titres during the initial few days after injection (~7-8 log per ml of serum or gram of tissue), dropping to 4-5 log over the next several days and then maintained at that level for the remainder of the infected animal's life^{1,3,11}. In contrast, viral nucleic acid sequences are barely detectable in tissues during this initial phase of heightened viral replication but accumulate steadily during the infection¹¹, suggesting that incomplete or defective viruses have formed *in vivo*. During persistence, antibodies to all LCMV proteins are made^{12,13} but

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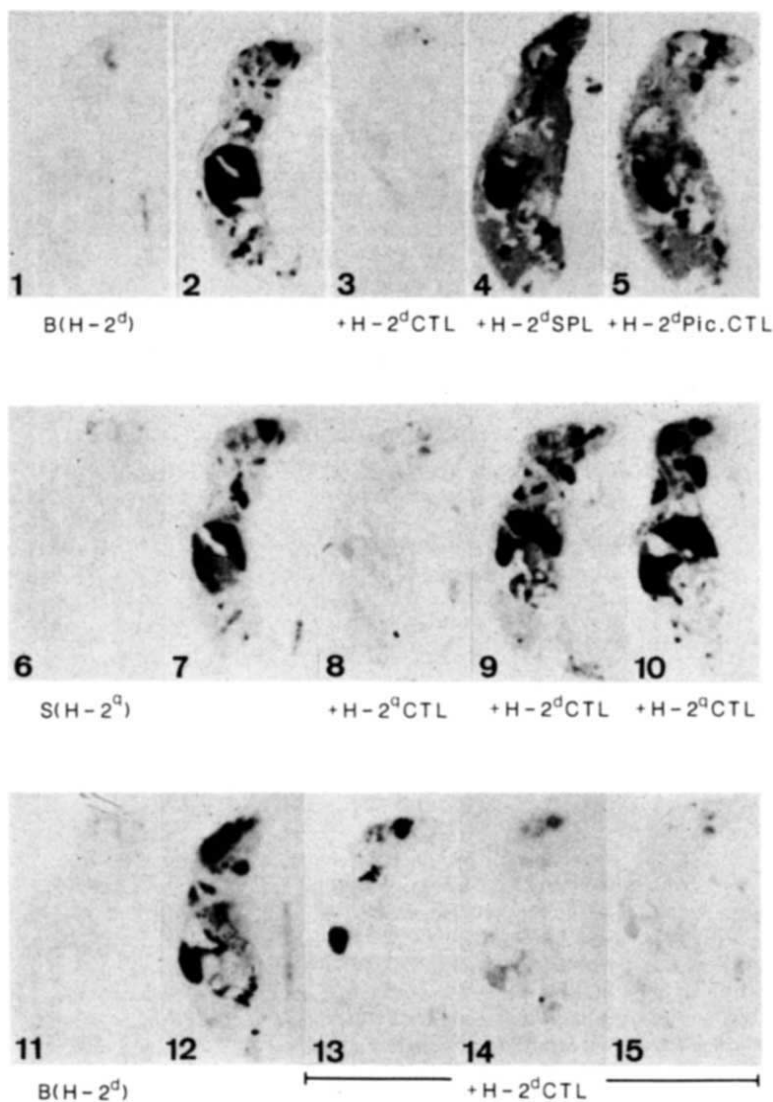


Fig. 1 Immunotherapy clears viral nucleic acid sequences and infectious virus from the persistently infected host. Clearance follows adoptive transfer of lymphocytes primed against the persisting virus (panels 1–5, 6–8, 12–15) into an H-2-compatible infected host (panels 6–10). Clearance of viral nucleic acid sequences from brain and kidney takes longer than from other tissues (panels 11–15). Mice were killed when 4–6 months old and 120 days after adoptive transfer of lymphocytes (panels 3–5, 8–10 and 15), except panels 13 and 14 which record studies 15 and 30 days, respectively, after cell transfers. Cells transferred were splenic lymphocytes (5×10^7 cells) collected from mice primed (1×10^4 PFU) 30–60 days earlier with either LCMV or Pichinde arenaviruses. All mice were treated identically and prepared for nucleic acid hybridization using a 545-base pair (bp) 32 P-labelled, nick-translated cDNA probe specific for the 5' S RNA of LCMV (Armstrong CA 1371 strain)^{11,35}. The titre (PFU) of infectious virus was determined for mouse serum using plaque assays on Vero cells¹⁰ ($\log_{10}$ PFU ml⁻¹): panel 2, 4.7; 3, <1.6; 4, 4.2; 5, 4.1; 7, 4.7; 8, <1.6; 9, 4.7; 10, 4.5; 12, 4.1; 13, 2.1; 14, <1.6; 15, <1.6. Negative controls: uninfected BALB/WEHI ($H-2K^d$, panels 1, 11) and SWR/J ($H-2K^q$, panel 6) mice. Positive controls: LCMV persistently infected (VC, virus carrier) BALB/WEHI (panels 2 and 12) and SWR/J (panel 7) mice. Persistent infection was initiated by inoculating newborn mice intracerebrally with 60 PFU of virus^{10,23}. Evidence for virus specificity in successful immunotherapy: panel 1, uninfected BALB/WEHI (B) mice; panels 2–5, BALB/WEHI VC. Panel 2, no treatment; panel 3, adoptive transfer of LCMV-specific H-2-restricted lymphocytes; panel 4, adoptive transfer of nonimmune H-2-restricted lymphocytes; panel 5, adoptive transfer of Pichinde-specific (Pic.) H-2-restricted lymphocytes. Evidence for H-2 restriction in successful immunotherapy (panels 6–10): panel 6, uninfected SWR/J (S) mice; panel 7, SWR/J VC; panel 8, SWR/J VC receiving LCMV-specific H-2-restricted lymphocytes; panel 9, SWR/J ($H-2^q$) VC receiving LCMV-specific H-2^d (BALB/WEHI) lymphocytes (such H-2^d lymphocytes cleared viral nucleic acid sequences from BALB/WEHI ($H-2^d$) VC—see panels 3, 15); panel 10, BALB/WEHI ($H-2^d$) VC receiving LCMV-specific H-2^q (SWR/J) lymphocytes (such H-2^q lymphocytes cleared viral nucleic acid sequences from SWR/J ($H-2^q$) VC—see panel 8). Kinetics of clearance of viral nucleic acid sequences (panels 11–15): panel 11, uninfected BALB/WEHI; panel 12, BALB/WEHI VC; panels 13–15, BALB/WEHI VC at 15 (panel 13), 30 (panel 14) or 120 (panel 15) days after receiving LCMV-specific H-2-restricted lymphocytes. Each experimental group contained 3–5 mice and similar results were obtained on analysis of other individual mice. SPL, non-primed splenic lymphocytes.

the virus-specific H-2-restricted CTL response is decreased^{1–3,10}, a selective decrease which suggests that at least one specific defect enables infection to persist. To test this hypothesis, we have reconstituted mice persistently infected with LCMV (virus carriers) with LCMV-specific CTL and studied the clearance of viral nucleic acid sequences, viral proteins and infectious virus. Acute immunopathological injury leading to death¹⁴ resulted on transfer into persistently infected mice of either $>3 \times 10^6$ cloned LCMV-specific CTL or splenic lymphocytes recovered from syngeneic mice primed 30–60 days previously with LCMV (memory/immune lymphocytes) and stimulated *in vitro* with LCMV-infected cells expressing class I and class II MHC antigens. Therefore, for our studies we used fresh immune lymphocytes obtained 30–60 days after priming.

Mice persistently infected with LCMV and receiving lymphocytes primed against LCMV (Fig. 1, panels 3, 8 and 13–15) clear both viral nucleic acid sequences and infectious virus. Identity between the H-2 type of the adoptively transferred LCMV-primed lymphocytes and the persistently infected animal is required for virus material to be cleared (Fig. 1, panels 3, 8, 13–15). In the absence of H-2-compatibility (Fig. 1, panels 9, 10), clearance does not occur. Further, transfer of lymphocytes from either unimmunized recipients (Fig. 1, panel 4) or lymphocytes primed with a different virus, Pichinde (Fig. 1, panel 5), fails to clear viral nucleic acid sequences or infectious virus. Pichinde, like LCMV, is a member of the arenavirus family but Pichinde-specific CTL do not recognize LCMV-infected targets

and vice versa^{15,16}. To examine more closely the H-2-restriction requirement, LCMV-specific memory splenic lymphocytes obtained from BALB/WEHI mice ($H-2K^d, I^d, D^d, L^d$) were transferred into either BALB/WEHI ($H-2K^d, I^d, D^d, L^d$), B10.A ($H-2K^k, I^k, D^d, L^d$) or SWR/J ($H-2K^q, I^q, D^q, L^q$) persistently infected mice. In two experiments (3–4 mice per group), within 30 days of lymphocyte transfer, a 2–3 log decrease in infectious virus from that usually carried in the sera during infection was observed in B10.A and BALB/WEHI mice but not in SWR/J mice. These results confirm and extend *in vitro* studies^{17,18} and suggest that clearance of viral materials is restricted primarily to class I MHC genes at the D^d, L^d locus.

Table 1 summarizes two of four experiments determining the phenotype of the effector lymphocyte responsible for clearance. Adoptive transfer of lymphocytes from which those bearing the Thy 1.2 or Lyt 2.2 markers had been removed^{19–21} prevents clearance of viral materials. In contrast, depletion of L3T4-bearing lymphocytes most often had a minimal effect on clearance. But some caution in interpreting these results is required as one of the monoclonal antibodies used to deplete this subset does not bind as well to complement as reagents used for Lyt 2.2 cell depletion^{19–21}. Nevertheless, in conjunction with the use of H-2 recombinant inbred mice to map the location of genetic elements responsible for viral clearance, these results indicate that immune elimination of viral materials is mediated primarily by virus-specific, MHC class I-restricted CTL^{7,17}.

Unexpectedly, clearance of viral materials from the CNS is

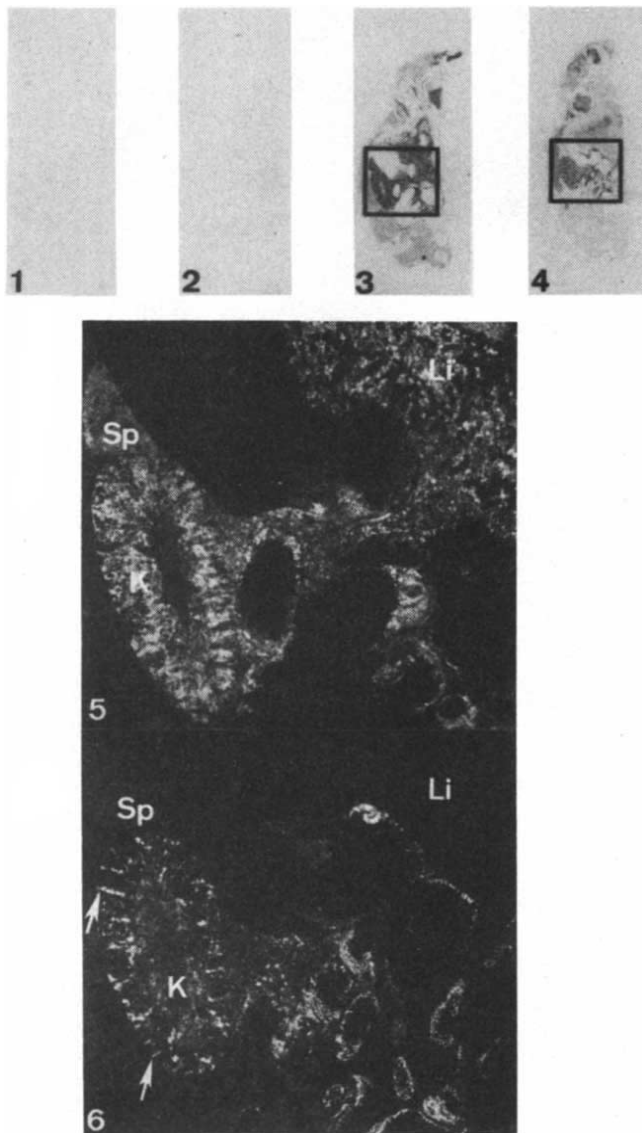


Fig. 2 Immunotherapy is associated with differential clearance of viral materials from various tissues of persistently infected BALB/WEHI mice. Six-week-old persistently infected mice (4–30 mice per group) received 5×10^7 splenic lymphocytes from H-2-restricted (syngeneic) mice primed 30–60 days earlier with 1×10^4 PFU of LCMV. Mice were killed 60 or 120 days after adoptive lymphocyte transfer. Titre of infectious virus was assayed by plaque formation on Vero cells¹⁰. The values shown below represent the mean of 4–5 mice per group, and results were equivalent in additional experiments.

	PFU g^{-1} or ml^{-1}	Day: 0	60	120
Serum	4.5	<1.6	<1.6	<1.6
Spleen	5.9	<1.6	<1.6	<1.6
Liver	5.2	<1.6	<1.6	<1.6
Lung	4.8	<1.6	<1.6	<1.6
Brain	5.2	3.0	1.9	1.9
Kidney	6.2	4.1	3.1	3.1

Expression of viral proteins (panels 1–6) in the whole animal was performed as described elsewhere²². Briefly, 40- μ m sections initially cut in a cryomicrotome, picked up on 3M tape¹¹ and transferred to biondyne nylon membrane, were reacted sequentially with monospecific guinea pig antibody against the three structural proteins encoded by the S RNA segment of LCMV (for 3 h at room temperature), a rabbit immunoglobulin G against guinea pig immunoglobulin (for 1 h at room temperature) and ^{125}I -labelled *Staphylococcus* protein A (for 1 h at room temperature; 5×10^5 – 1×10^6 c.p.m. ml^{-1}). Panel 1 is a section from an uninfected mouse reacted with all the reagents. Panel 2 and 3 are sections from a mouse persistently infected with LCMV. Panel 2 has not been reacted with the guinea pig antibody to LCMV but has received the rabbit antibody to guinea pig immunoglobulin and ^{125}I -labelled *Staphylococcus* protein A. All three reagents have been used in panel 3. Panel 4 is from a mouse sectioned 60 days after adoptive transfer of virus-specific H-2-restricted lymphocytes. Panels 5 and 6 are enlargements ($\times 5$) of the boxed areas in panels 3 and 4, respectively. Panels 3 and 4 show that 60 days after adoptive transfer of immune lymphocytes, dramatic clearance of virus materials had occurred in most tissues, that is, spleen, liver, lung, heart and salivary gland but not in brain or kidney (also see Fig. 1 for nucleic acid clearance). Panels 5 and 6 show clearance of viral materials in spleen (Sp) and liver (Li), and record the change in viral protein distribution in kidney (K). Arrows indicate an area containing glomeruli where cleared viral materials have been trapped in the form of immune complexes (see Fig. 3, panel 9). Accumulation of immune complexes in the intestinal tract was also noted. Similar results were observed with persistently infected SWR/J mice receiving immunotherapy.

Table 1 Adoptive transfer of LCMV-specific, H-2-restricted Lyt 2⁺ T lymphocytes clears infectious virus from persistently infected mice

Experiment no.	Treatment of immune lymphocytes used for adoptive transfer	$\log_{10}$ PFU per ml of sera in LCMV-infected mice*			% Specific ^{51}Cr released <i>in vitro</i> from target cells infected with†:				
		Immune cells sensitized to:			LCMV				
		LCMV	Pich.	LCMV	50:1 ⁴	25:1	Pich. 50:1	Uninf. 50:1	LCMV 50:1
		Syngeneic			H-2-restricted			Allogenic	
1	Nil	1.6±0.0	4.2±0.4	4.6±0.5	84	67	5	2	4
	Antibody to Lyt 2.2 (AD4.15)	4.1±0.3	ND	ND	0	2	ND	4	ND
	Antibody to L3T4 (GK1.5)	2.4±0.5	ND	ND	76	61	3	2	ND
	Antibody to Thy 1.2	4.6±0.4	ND	ND	5	4	ND	4	ND
	Virus titre before cell transfer	4.5±0.5	ND	ND					
2	Nil	1.6±0.0	ND	ND					
	Antibody to Lyt 2.2 (YTS 169.4)	3.2±0.5	ND	ND					
	Antibody to L3T4 (YTS 191.1)	1.8±0.3	ND	ND					
	Virus titre before cell transfer	3.8±0.4	ND	ND					

Mice had been persistently infected since birth with LCMV as described elsewhere^{10,23}. Immune spleen cells specific for LCMV or Pichinde (Pich.) were transferred into mice persistently infected with LCMV. Mice infected with either virus do not generate cross-reactive CTL. Adoptive transfers used 5×10^7 immune lymphocytes and the methodology for *in vitro* ^{51}Cr release assay is published elsewhere^{10,15}. The monoclonal antibodies used to delete lymphocyte subsets have been reported elsewhere^{19–21}. These antibodies were used with rabbit sera (1:10 dilution) as a source of complement.

* Values are mean $\pm$ s.d. (4–5 mice per group).

† Companion *in vitro* CTL assay using splenic lymphocytes from different mice acutely primed 6–7 days previously with 1×10^5 PFU (plaque forming units) of LCMV or Pichinde^{10,15,27}, at various effector/target cell ratios. Uninf., uninfected; ND, not determined.

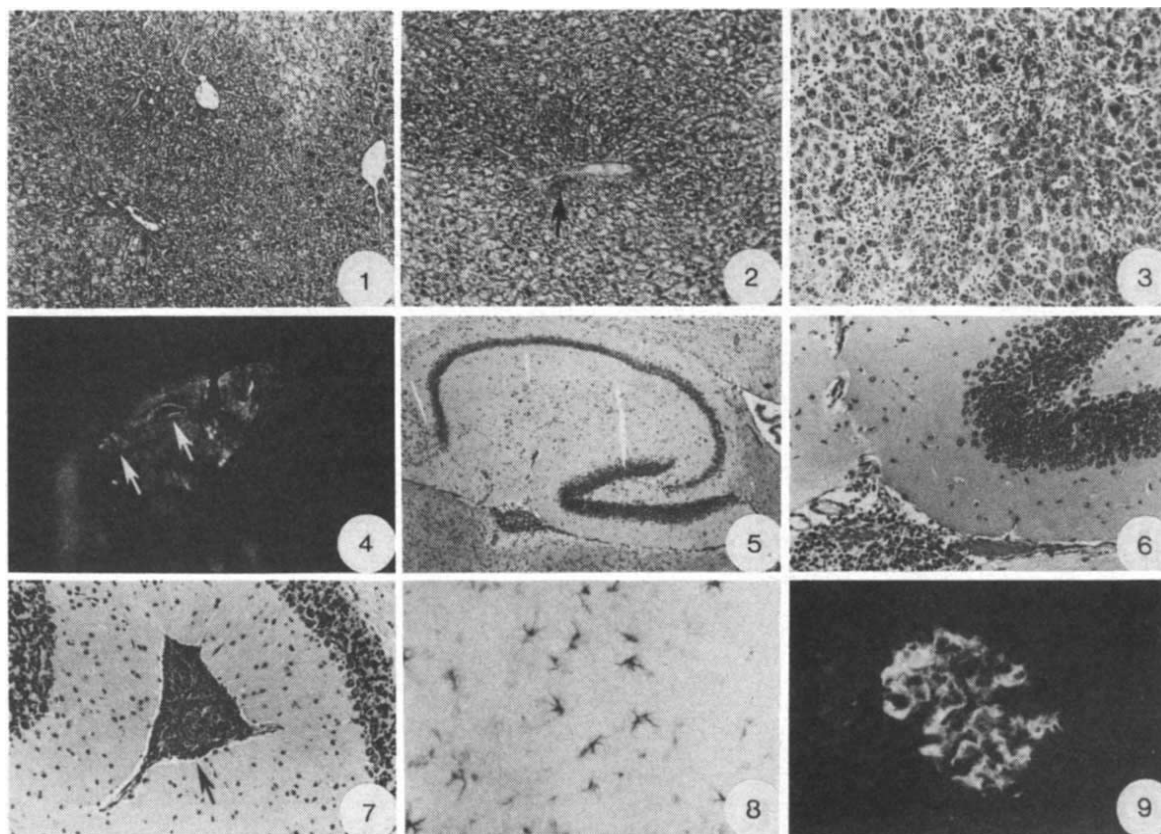


Fig. 3 Histological and immunochemical analysis of tissues obtained from mice persistently infected with LCMV and receiving immunotherapy to clear viral materials. Panels 1–3, photomicrographs of liver sections ($\sim \times 250$) demonstrating accumulation of inflammatory exudates and necrosis associated with clearance of viral materials (see Figs 1, 2). Panel 1, LCMV-infected mouse 15 days after adoptive transfer of LCMV-specific H-2-restricted lymphocytes that had been pretreated with antibody to Thy 1.2 and complement. Note the lack of inflammatory response. Equivalent results were obtained with transfer of either non-H-2-restricted or non-LCMV-immune lymphocytes or treatment of immune lymphocyte with anti-Lyt 2.2 antibody and complement before adoptive transfer. Panel 2, LCMV-infected mouse 5 days after adoptive transfer of LCMV-specific H-2-restricted lymphocytes. Arrow indicates early lymphocyte infiltration. Panel 3, similar to panel 2 but 15 days after adoptive transfer. Note the massive lymphocyte infiltration and destruction of hepatic cells. Panel 4, protein blot of CNS of a 10-week-old SWR/J mouse with persistent LCMV infection showing localized LCMV material. Arrows point to viral proteins in the cerebellum (left) and the dentate and hippocampal gyrus (right). Methods for protein blotting are given in ref. 22 and Fig. 2 legend. This is the usual LCMV protein distribution in persistently infected mice. Panels 5, 6, low ($\sim \times 60$) and high ($\sim \times 250$) magnification of the dentate and hippocampal gyrus 15 days after adoptive transfer of LCMV-specific H-2-restricted lymphocytes. Note the preservation of normal cellular architecture, and the absence of infiltration in the brain parenchyma and absence of neuronal necrosis. At electron microscopic level, neurones of dentate show normal cytomorphology. Tissue taken from the same mouse whose liver is shown in panel 3. Panel 7, accumulation of inflammatory cells (arrow) in the leptomeninges with neither infiltration into the cerebellum, alteration of cerebellum architecture nor necrosis of neurones in the cerebellum. Tissue taken from the same mouse whose tissues are shown in panels 3, 5 and 6 ($\times 250$). Panel 8, cytomorphologically normal section taken at the region of the dentate gyrus from a mouse persistently infected with LCMV and subjected to adoptive transfer of 5×10^7 LCMV-specific H-2-restricted lymphocytes. The section was stained with a monospecific antibody to glial fibrillary acid protein⁴⁶. Note the accumulation of activated glia, in the area of normal neurone distribution ($\times 300$). Panel 9, heavy accumulation of immune complexes in the glomerulus of a 104-day-old BALB/WEHI mouse with persistent LCMV infection since birth which was subjected to adoptive transfer of LCMV-specific H-2-restricted lymphocytes at 6 weeks old. Note the heavy concentration of host immunoglobulin in the glomerulus. Immunochemical staining used a rhodamine-conjugated rabbit immunoglobulin to mouse IgG on an ether/alcohol (1:1) and 95% ethanol-fixed 5- μ m section^{12,23}. Results were similar in at least four additional animals studied. In contrast, 104-day-old BALB/WEHI mice persistently infected with LCMV not subjected to adoptive cell transfer showed only trace to light amounts of trapped immunoglobulin⁴⁷ (data not shown).

distinct in pattern and timing compared with clearance from other organs. For example, Fig. 1, panels 12–15 shows that virus materials are cleared from most organs (liver, lungs and spleen) 15 days after adoptive transfer of virus-specific H-2-restricted lymphocytes but there is no corresponding clearance in either CNS or kidney tissues. Viral nucleic acid sequences remain in kidney and CNS 30 days after transfer (Fig. 1, panel 14), and only after 120 days is clearance complete in the CNS (panel 15). Overall, 12 of 16 (75%) mice showed significant clearance of virus materials from livers, lungs, spleens and sera by 15 days after transfer. In comparison, clearance usually did not begin in the brain until 30–60 (3 of 16, 19%) days after transfer, and even then clearance was less complete than in other organs. By

120 days after transfer, 13 of 15 (86%) mice had cleared viral materials from their brains as well as livers, lungs, spleens and sera.

Figure 2 provides data on the clearance of infectious virus and viral proteins following adoptive transfer of LCMV-specific H-2-restricted lymphocytes. Infectious virus was effectively cleared (not detectable) in the serum, spleen, liver or lung 60 days after adoptive transfer. In contrast, although reduced by 2 log units, infectious virus remained in the brains and kidneys of these mice. Similar results were obtained when virus-specific antibodies and ¹²⁵I-labelled *Staphylococcus* protein A were used (Fig. 2, panels 3–6); this technique detected 5–20 ng of viral protein in a 40- μ m section of whole body *in situ*²². Again,

clearance of viral proteins from the spleen, liver and lung was dramatic, but viral protein remained in the CNS and kidney (Figs 2, 3, panels 3–6). The presence of viral proteins in the kidney 60 days after adoptive transfer suggests the formation of virus-antibody immune complexes (Fig. 2, arrows in panel 6) trapped in the renal glomerular filter; this was confirmed (Fig. 3, panel 9) by immunohistochemistry²³ and indicated further by the accumulation of immune complexes in the gut wall (Fig. 2, panel 6).

As clearance of viral materials is primarily dependent on effector lymphocytes that are Thy 1.2⁺ Lyt 2⁺, virus-specific and MHC class I-restricted, we expected to see evidence of CTL activity, that is, inflammation, cell lysis and tissue necrosis^{24–27}. As anticipated, the liver (Fig. 3, panels 1–3), lung and kidney showed infiltration and necrosis. In contrast, multiple sections of CNS tissues harvested from similarly treated mice and studied sequentially from 5 to 120 days after adoptive lymphocyte transfer failed to show lymphocytic infiltration into the brain parenchyma or necrosis of brain tissue (Fig. 3), despite clearance of virus materials from the CNS (Fig. 1). As neuronal cells—cells that contain viral materials²⁸—do not regenerate, their loss or destruction should have been evident. This is especially the case in the dentate gyri and cerebellum Purkinje layers, where the majority of neurones contain LCMV antigens²⁸ (Fig. 3). In preliminary studies, counting of neurones revealed no significant loss in persistently infected mice either untreated or subjected to adoptive transfers (P.W.L., unpublished observations). The absence of dead neuronal cells or CNS destruction in persistently infected mice following viral clearance presents a totally different picture from that seen in other organs (Fig. 3) and correlates with the lack of clinically detectable CNS injury in such mice. Detection of glial fibrillary acidic protein (Fig. 3, panel 8) revealed activation of glial cells in the absence of CNS cell lysis. Thus, CTL may not exert their usual lytic effect *in vivo* on components of the CNS; the reason for this is unclear, although there are reports that H-2 class I gene products are minimally expressed on cells in the CNS^{29–31}, especially neurones, the principal CNS cells in which LCMV products accumulate²⁸. This inability to kill a highly differentiated important host cell, incapable of being replaced, has an obvious selective advantage.

For CTL to function lytically or to release products such as

lymphokines, they need to recognize the specific priming antigen. Lymphocytes accumulate in the Virchow–Robbin space surrounding vessels supplying the brain and in the leptomeningeal area but not inside the brain parenchyma (Fig. 3). The inability of immune lymphocytes to accumulate in the brain is probably the reason for the absence of direct CTL-mediated injury. The accumulation of effector cells in the Virchow–Robbin spaces suggests, however, that appropriate viral antigens in association with H-2 molecules are being recognized here. This could lead to the release of a soluble factor by the CTL, that is, a lymphokine that can cross the blood–brain barrier and act on LCMV-infected neurones. The clearance of viral materials in the absence of cell lysis resembles, in part, observations in systems using interferon or similar lymphokines *in vitro* (reviewed in refs 32–34). We do not know how lymphokines alone or in combination with other factors such as antibodies perform this task, although effects of interferon-like material on transcription, translation, glycosylation and budding have been recorded^{32–34}. From our knowledge of LCMV replication and transcription^{11,35,36}, we hypothesize that the postulated antiviral factor probably destabilizes the viral ribonucleoprotein complex. Further studies are in progress.

Finally, the clearance of viral materials (infectious virus, viral nucleic acid sequences and proteins) from several organs of persistently infected mice probably occurred by reconstitution of LCMV-specific CTL that malfunctioned or had been deleted during the virus infection. Our studies extend earlier ones by Volkert³⁷ in that they demonstrate the phenotype of the immune lymphocyte involved and the clearance of viral nucleic acids. Recently, reports on a variety of DNA and RNA viruses describe tropism for lymphocytes and their various subsets^{24,38–43}. Several virus infections of lymphocytes have been shown to abrogate or modulate specific lymphocyte functions, such as cytotoxic lymphocyte killing^{10,38,44,45} or production of antibodies^{38,43}. Hence, it is likely that the principles of clearance for persistent viral infection described here may be effective for the treatment of other persistent microbial infections or malignancies involving a selective defect in lymphocyte function.

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A new mechanism for the neutralization of enveloped viruses by antiviral antibody

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Despite the considerable research that has been carried out into viral neutralization by antiviral antibody, its mechanisms remain poorly understood¹. Cases have been reported in which antiviral antibody can inhibit viral replication without inhibiting the binding and uptake of virus by susceptible cells¹. It has been shown that many enveloped viruses enter their target cells by endocytosis and are subsequently located in cellular compartments of increasing acidity². With several enveloped viruses this acidic pH can catalyse a fusion reaction between the membrane of the virus particle and that of a prelysosomal endosome, thus enabling the viral core to enter the cytosol and replication to commence^{2,3}. We have recently demonstrated that such an endosomal fusion event at mild acidic pH is involved in the entry pathway of the enveloped flavivirus, West Nile virus (WNV), into macrophages⁴⁻⁶. We now show that antiviral antibody can neutralize WNV by inhibiting this intra-endosomal acid-catalysed fusion step and we speculate on possible implications for the future design of antiviral vaccines.

The *in vitro* replication of WNV can be enhanced up to 100-fold in the macrophage-like cell line P388D1 if infection is carried out in the presence of subneutralizing concentrations of antiviral antibody⁷. This is due to an increased interaction between virus particles, coated with subneutralizing concentrations of antibody, and Fc receptors on the cell surface^{4,8}. WNV enters P388D1 cells very rapidly at 37 °C, both in the absence and presence of optimally 'enhancing', subneutralizing concentrations of antiviral antibody^{4,5}. Within 1-3 min of endocytosis, virus particles appear in prelysosomal endosomes, from where the viral core enters the cytosol due to acid pH-dependent fusion between viral and endosomal membranes^{3,6}. Unfused virus particles start to appear in lysosomes, where they undergo hydrolytic degradation from 10 min after the start of internalization^{4,5}.

The aim of the present study was to quantify the fusogenic intracellular removal of the lipid envelope from WNV particles on synchronized internalization into P388D1 cells, and to examine the effects of various concentrations of antiviral antibody both on this process and on viral infectivity. To measure viral uncoating, ³H-uridine-labelled purified WNV particles were synchronously internalized into P388D1 cells and the cells were then mechanically lysed and the RNase-degradable ³H radioactivity was quantified (see Fig. 1 legend). The lipid envelope normally protects viral RNA from hydrolysis by added RNase and only the RNA of uncoated virus particles is accessible to added RNase and can thus be degraded.

Before internalization at 37 °C, ³H-WNV particles were initially bound to the P388D1 cell surface at 0 °C (when internalization does not occur) in the presence of a concentration (1 µg ml⁻¹) of purified polyclonal immune antiviral IgG which had previously been shown to enhance maximally both the binding and replication of WNV particles. This step was necessary because in the absence of antibody WNV binds poorly to P388D1 cells⁴ and insufficient c.p.m. were bound to the cells for significant results to be obtained in the present study. The entry pathway of WNV into P388D1 cells, however, has previously been shown to be identical in the absence and presence of 'enhancing' concentrations of antiviral antibody⁵.

After the initial binding of virus particles at 0 °C (with 1 µg ml⁻¹ immune IgG), the cell-surface virus particles were treated for 30 min or 2 h, still at 0 °C, with various concentrations of purified immune or non-immune rabbit IgG (0-

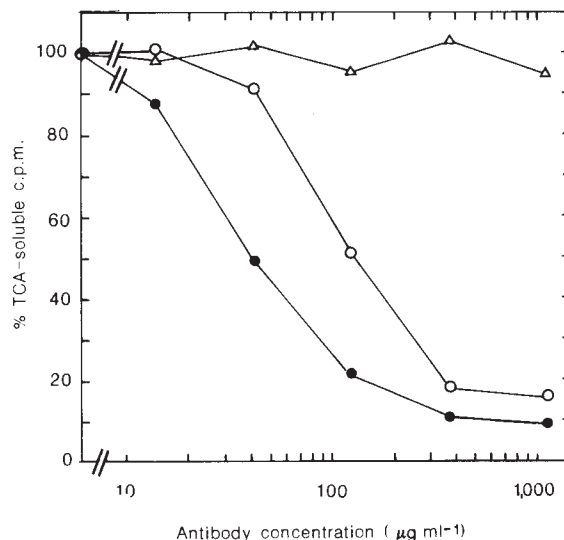


Fig. 1 Effect of immune and non-immune IgG on the uncoating of WNV pre-bound to P388D1 cells. ³H-uridine-labelled WNV particles (purified on sucrose gradients as in ref. 6; 7×10^3 particles per c.p.m.) in Leibowitz L15 medium with 15 mM HEPES, 0.2% bovine serum albumin, pH 7.6 (B-medium) were prebound at 0 °C for 1 h to monolayers of P388D1 cells in 35-mm diameter round plastic Petri dishes (Falcon), in the presence of 1 µg ml⁻¹ anti-WNV polyclonal immune IgG derived from a New Zealand White rabbit and purified by protein A-Sepharose affinity chromatography. The cells were then washed twice with ice-cold fresh B-medium and incubated with various concentrations of the same stock of purified immune IgG at 0 °C for 0.5 h (○) or 2 h (●), or with various concentrations of purified non-immune IgG for 2 h (△). The cells were then washed twice at 0 °C and pre-warmed (37 °C) B-medium was added to cells to initiate internalization of virus. Synchronized internalization was allowed to proceed at 37 °C for 15 min, at which time previous experiments (not shown) had indicated that viral uncoating was complete. The medium was then aspirated and cells were cooled by addition of 1.0 ml of ice-cold H-buffer (0.01 M triethanolamine, 0.01 M acetic acid, 0.25 M sucrose, pH 7.5). Cells were scraped from plates, lysed mechanically by passage through a 25-gauge syringe needle, and the amount of uncoated viral ³H-RNA determined by measuring its susceptibility to degradation to TCA-soluble material by added RNase, essentially as described in ref. 11. The uncoating occurring after treatment of pre-bound virus particles with various concentrations of immune and non-immune IgG is expressed as a percentage of the uncoating occurring after treatment of pre-bound virus particles with buffer only. The latter was regarded as 100% for this figure. This 100% actually represents 400 TCA-soluble c.p.m., which is 27% of the c.p.m. initially bound to each P388D1 cell monolayer.

1,020 µg ml⁻¹). Cells were then washed and synchronized and viral internalization was allowed to occur by warming to 37 °C.

Under these conditions, immune IgG reduced viral uncoating in a concentration-dependent fashion (Fig. 1). The concentrations needed for 50% inhibition of uncoating were 129 µg ml⁻¹ (for 30 min treatment) or 40 µg ml⁻¹ (for 2 h treatment). Immune IgG reduced viral infectivity under these conditions, also in a concentration-dependent fashion, with a potency that closely paralleled the inhibition of uncoating (Fig. 2). Viral infectivity was neutralized by 50% at 90 µg ml⁻¹ (30 min treatment) or 30 µg ml⁻¹ (2 h treatment). Non-immune IgG had no effect on either viral uncoating or infectivity.

Control experiments using purified ³⁵S-methionine-labelled WNV (in which most radioactive label is located in the E membrane glycoprotein⁴) showed that these inhibitory effects did not result from immune IgG causing WNV particles to elute from the P388D1 cell plasma membrane (Fig. 3). These experi-

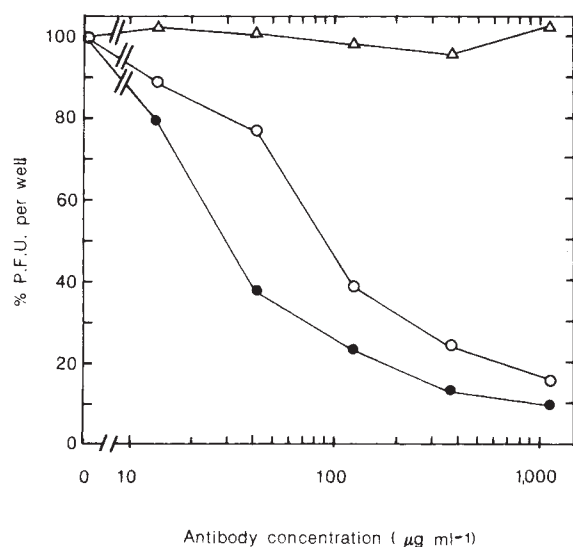


Fig. 2 Effect of antiviral antibody on the infectivity of WNV pre-bound to monolayers of P388D1 cells. WNV particles were pre-bound at 0 °C in B-medium for 1 h to monolayers of P388D1 cells in the presence of 1 $\mu\text{g ml}^{-1}$ of immune IgG. The cells were then washed twice in ice-cold B-medium and incubated with various concentrations of immune IgG for 0.5 h (○) or 2 h (●) or with various concentrations of non-immune IgG for 2 h (△). The cells were then washed twice at 0 °C and pre-warmed (37 °C) B-medium was added to allow internalization. After 1 h at 37 °C the cells were washed twice again and growth medium containing 0.75% carboxymethylcellulose was added. Virus was allowed to replicate for 3 days at 37 °C and plaques were then counted. The number of plaques formed after treatment of pre-bound virus with the various concentrations of immune and non-immune IgG is expressed as a percentage of plaque-forming units (PFU) found after treatment of pre-bound virus with buffer only. The latter was regarded as 100% for this figure and represents 95 plaques per well.

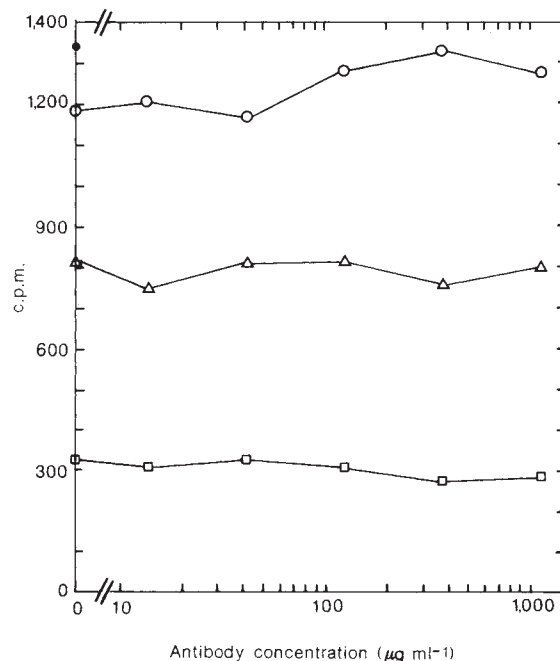


Fig. 3 Effect of immune antiviral IgG on the cell association and lysosomal degradation of WNV pre-bound to P388D1 cells. Purified ^{35}S -methionine-labelled WNV particles were pre-bound to P388D1 cell monolayers in the presence of 1 $\mu\text{g ml}^{-1}$ of immune IgG for 1 h at 0 °C and then incubated at 0 °C for 0.5 h with various concentrations of immune IgG. Cells were washed with B-medium and synchronized viral internalization was carried out for 90 min at 37 °C. ●, the amount (1,340 c.p.m.) of ^{35}S -WNV initially bound to the P388D1 monolayers; ○, the amount of WNV bound to monolayers after treatment of pre-bound WNV with various concentrations of immune IgG for 0.5 h at 0 °C; □, the cell-associated c.p.m. after synchronized internalization for 90 min at 37 °C; △, the TCA-soluble c.p.m. in the medium after synchronized viral internalization for 90 min at 37 °C.

ments also showed that cell-surface WNV particles were endocytosed and that viral glycoproteins reached the lysosomal compartment where they underwent degradation to trichloroacetic acid (TCA)-soluble material, regardless of whether or not the virus particles bound to the cell surface had been preincubated with neutralizing concentrations of antibody. Similarly, time-course experiments up to 2.5 h after warming to 37 °C demonstrated that the degradation of virus particles followed similar kinetics, whether or not neutralizing antibody had been incubated for 2 h with cell-surface virus particles (not shown). Furthermore, capping of virus particles on the P388D1 cell surface, after warming to 37 °C, could not account for the viral neutralization seen, because in both the uncoating and infectivity studies (Figs 1, 2) there was less than one virus particle bound per cell (based on a knowledge of the number of ^3H -uridine-labelled virus particles per c.p.m. in viral preparations).

The similarities between antibody concentrations needed to inhibit intra-endosomal fusion-mediated uncoating and to neutralize viral infectivity suggest that viral neutralization results from the inhibition of viral membrane fusion. Any unfused virus particles will subsequently be transferred rapidly to lysosomes and inactivated by hydrolysis. In agreement with this, pretreatment of WNV particles with immune IgG inhibited WNV fusion with artificial liposomes in a concentration-dependent manner at pH 6.6 (Fig. 4).

Various mechanisms can be proposed to explain how immune IgG might prevent acid pH-dependent fusion of WNV with target membranes. Antibody might simply sterically block an interaction between the target membrane and a region of the

WNV E membrane glycoprotein responsible for bringing about fusion. If a hydrophobic epitope is displayed on the WNV E glycoprotein at low pH, and this inserts into a target membrane bilayer to bring about fusion (as has been proposed for orthomyxoviruses³), neutralizing antibody binding on or near this epitope might make it too strongly charged to interact with the target lipid bilayer. Alternatively, if a particular antibody idiotype binds to the E glycoprotein at a particular critical location at neutral pH, this might stabilize the glycoprotein and prevent a conformational change (necessary for fusion) when the pH is lowered.

As a general principle, the success of an antiviral vaccine might be increased if it could stimulate the production of antibody idiotypes primarily directed against functionally important regions of the viral surface and not against non-neutralizing viral epitopes. More particularly, for several viruses, most notably the flavivirus Dengue virus, the presence of non-neutralized antibody-coated virus particles in an individual's circulation has been implicated in causing a more severe form of disease^{9,10}. This is probably the result of increased viral replication in cells of the monocyte-macrophage lineage, due to the interaction of antibody-coated, non-neutralized virus particles with cell-surface Fc receptors.

After virus particles have been endocytosed into their host cell, they are paradoxically in an extremely vulnerable position, because agents which can inhibit the acid-driven fusion between virus particles and the endosomal membrane will cause the virus to be inactivated. Synthetic peptide and anti-idiotypic vaccines have the potential to allow small portions of viral surface gly-

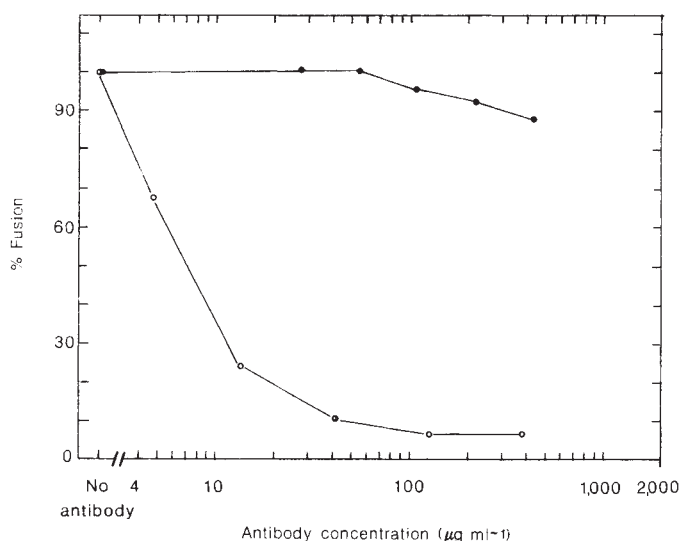


Fig. 4 Inhibition of fusion of WNV with liposomes by antiviral immune IgG. ^3H -uridine-labelled WNV (6,000 c.p.m.) was preincubated with various concentrations of immune or non-immune IgG for 2 h at 0 °C and pH 7.2. Liposomes composed of cholesterol, phosphatidylcholine, phosphatidylethanolamine and sphingomyelin (molar ratio 1.5:1:1:1), containing 2 mg ml $^{-1}$ of RNase, were added (final lipid concentration 0.1 mM) and the pH was lowered to 6.6. Mixtures were incubated for 2 min at 37 °C and then TCA-soluble ^3H radioactivity was determined. The quantity of TCA-soluble RNA is a measure of the amount of fusion-mediated viral uncoating that has taken place, thus allowing RNA in the viral core to be degraded (see ref. 6). The final amount of TCA-soluble ^3H radioactivity obtained with each antibody concentration is expressed as a percentage of that obtained with WNV preincubated in the absence of antibody (which is 100% here and actually represented 40% of the initial total input of radioactivity). ○, Immune IgG; ●, non-immune IgG.

coproteins to be presented as an immunological stimulus. If vaccines could be developed which stimulated the production of antibodies that neutralized an enveloped virus by binding specifically to a particular functionally important region of the viral surface that was responsible for pH-dependent fusogenic uncoating, this would lessen the chances of the presence of non-neutralized virus-antibody complexes in an individual's circulation. The intracellular fusion step is crucial to infection of cells by several enveloped viruses and could thus be visualized as the 'Achilles heel' in the entry pathway of these viruses into cells. If specific antibody can cause failure of the viral core to penetrate fusogenically into the cytosol from a prelysosomal compartment, subsequent lysosomal degradation and inactivation of the virus particle will rapidly follow, as has been shown in the present study.

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A novel type of human papillomavirus associated with genital neoplasias

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The role of human papillomaviruses (HPVs) in the development of genital neoplasias has been well documented¹⁻⁵. The genomes of two HPV types, HPV16 and HPV18, have been found to be associated with about 70% of invasive carcinomas of the uterine cervix^{2,4,6}. As, under non-stringent hybridization conditions, HPV DNA sequences have been detected in about 90% of cervical carcinomas^{4,6}, it seems likely that additional HPV types are associated with these tumours. Here we report the molecular cloning and characterization of a novel HPV type, tentatively named HPV33, whose sequences have been detected in 4-8% of biopsies of genital intra-epithelial neoplasias and cervical invasive carcinomas, usually as free monomeric or oligomeric molecules. However, in one specimen of vulvar Bowen's disease, HPV33 DNA sequences were integrated in the host-cell genome. Thus, HPV33 probably represents an additional type of potentially oncogenic genital HPV.

While screening biopsies of invasive carcinomas of the uterine cervix for the presence of HPV genomes, we detected in a tumour DNA sample HPV sequences which cross-hybridized only under non-stringent conditions⁷ (T_m -40 °C) with a ^{32}P -labelled HPV16 DNA probe (Fig. 1A, lane a). Cleavage of the DNA preparation by *Bgl*II yielded an 8-kilobase (kb) fragment which is characteristic of all linearized HPV DNAs. The latter fragment was cloned in bacteriophage λ L47.1 (ref. 8) and subcloned in plasmid plink322 (ref. 9) (see Fig. 1 legend and ref. 10). After cleavage with a mixture of *Bgl*II and *Pst*I endonucleases, the recombinant DNA and the original DNA preparation yielded similar fragments totalling ~8 kb, as revealed by blot hybridization experiments using the purified cloned DNA as a probe (Fig. 1A, lanes b, c); this indicated that the entire HPV DNA sequence has been cloned. The novel HPV will henceforth be referred to as HPV33.

A restriction map of the cloned DNA was constructed as described previously¹⁰ (Fig. 2). This map differs from those of all the HPVs described so far (refs 4, 7, 10-22; R. S. Ostrow, personal communication; and M. Favre *et al.*, in preparation). Blot hybridization experiments were set up to determine the extent of homology between HPV33 DNA and the DNAs of HPVs 1-32 (refs 4, 7, 10-23). Blotted HPV DNAs, separated from the plasmid sequences, were hybridized with ^{32}P -labelled HPV33 DNA under stringent conditions (T_m -20 °C)^{7,20,22}. The labelled HPV DNA showed a low degree of cross-hybridization with HPV16 DNA, weak or very weak cross-hybridization with HPV6, 11, 13, 18, 27, 31 and 32 DNAs, and almost no cross-hybridization (or none at all) with the other HPV DNAs (data not shown). Therefore, it seems justified to consider HPV33 as a novel HPV type.

The sequence homology between HPV33 and HPV16 DNAs was studied further by heteroduplex mapping^{24,25} (see Fig. 3 legend): under stringent conditions (T_m -20 °C), three small duplex segments (corresponding to ~7% of the genome length) were observed on rare heteroduplex molecules (Fig. 3A). Under conditions of lower stringency (T_m -39 °C), HPV33 and HPV16 DNAs were paired over about 75% of their genome length (Fig.

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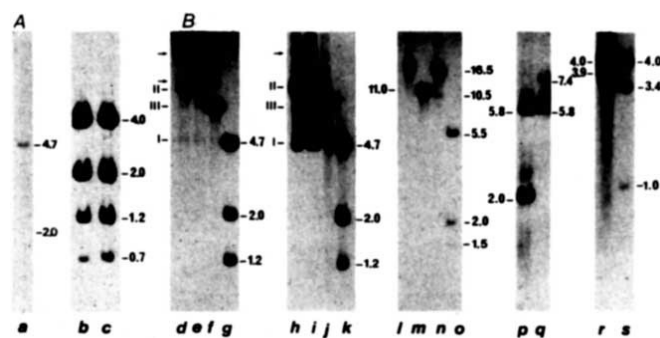


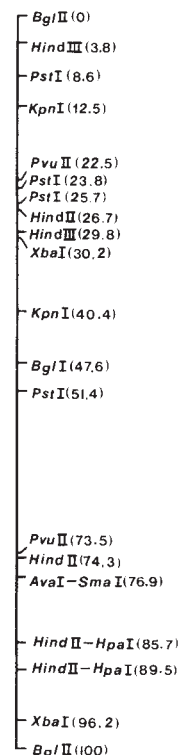
Fig. 1 **A**, Blot hybridization analysis of the HPV33 DNA sequences found in an invasive cervical carcinoma. The total cellular DNA extracted from a biopsy of a cervical cancer (10 µg) (lanes *a*, *b*) and a mixture of cloned HPV33 DNA from the same tumour (0.25 µg) and calf thymus DNA (10 µg) (lane *c*) were cleaved with either *Pst*I (*a*) or a mixture of *Bgl*II and *Pst*I (*b*, *c*). The hybridizations were performed either under non-stringent conditions (T_m -40 °C) with 32 P-labelled HPV16 DNA (*a*), or under stringent conditions (T_m -20 °C) using 32 P-labelled cloned HPV33 DNA (*b*, *c*). **B**, Analysis by blot hybridization of the physical state of HPV33 DNA in genital lesions. The DNA preparation used for molecular cloning of HPV33 DNA (10 µg) (lanes *d*-*g*), or the DNAs extracted from biopsies of a cervical intra-epithelial neoplasia (0.5 µg) (lanes *h*-*k*, *p* and *r*), and of a Bowen's disease of the vulva (5 µg) (lanes *l*-*o*, *q* and *s*) were either untreated (*d*, *h* and *l*) or treated with a mixture of *Bam*HI and *Eco*RI (*e*, *i* and *m*), with *Bgl*II (*f*, *j* and *n*), *Pst*I (*g*, *k* and *o*), *Hind*III (*p*, *q*) or *Pvu*II (*r*, *s*). Hybridizations were performed at T_m -20 °C, using 32 P-labelled HPV33 DNA. The sizes of the fragments are given in kb. The HPV33 DNA yielded an additional 0.16-kb *Pst*I fragment visible after a longer exposure time (lanes *b*, *c*, *g* and *k*). The migration of HPV33 DNA molecules form I, II and III is also shown. Arrows indicate oligomeric DNA molecules.

Methods. Total cellular DNA was extracted from biopsies³¹. The DNA preparations and purified cloned HPV33 DNA were treated with the appropriate restriction endonucleases (Amersham), and the cleavage products and the untreated DNA preparations were electrophoresed in vertical 1% agarose slab gels and transferred to GeneScreen Plus membranes (NEN). Hybridizations were carried out at 42 °C in 1 M NaCl, with 32 P-labelled HPV16 or HPV33 DNAs (specific activity 0.9-2 × 10⁸ c.p.m. µg⁻¹), in the presence of either 50% or 20% formamide, corresponding to stringent (T_m -20 °C) and non-stringent (T_m -40 °C) hybridization conditions, respectively. The hybrids were detected by autoradiography¹⁴. For the cloning of HPV33 DNA, the DNA extracted from a biopsy of an invasive cervical carcinoma was treated with *Bgl*II, a one-cut enzyme for HPV33 DNA, and the cleavage products were fractionated by centrifugation in a preformed 5-40% sucrose gradient^{20,22}. The DNA fragments of ~8 kb were introduced into the polylinker plasmid plink322 (ref. 9) at the *Bgl*II site. The recombinant molecules were cleaved with *Bam*HI (a no-cut enzyme for HPV33 DNA and a one-cut enzyme for plasmid plink322), inserted in bacteriophage λ L47.1 at the *Bam*HI site, then cloned in *Escherichia coli* K-12 strain LA 101 (ref. 32). The phages that had integrated the HPV33 DNA were identified by plaque hybridization³³ using 32 P-labelled HPV16 DNA, at T_m -40 °C. HPV33 DNA was purified from recombinant phage DNA molecules, and subcloned in plasmid plink322 (ref. 10).

3B). The regions of partial homology, yielding stable heteroduplex segments at T_m -39 °C and -26 °C, were mapped and the relative orientation of the HPV33 and HPV16 DNA^{19,26} maps was determined (Fig. 3C). The regions of strongest homology, detected at T_m -26 °C, were mapped to the 3' end of E1, the 5' end of E2, the 3' portion of L2 and the L1 open reading frames (ORFs) of the HPV16 genome²⁶ (Fig. 3C).

We searched for HPV33 DNA sequences in biopsies of cervical intra-epithelial neoplasias and invasive carcinomas (29 and 53 cases, respectively), intra-epithelial neoplasias of the external genitalia (34 cases of bowenoid papulosis and 17 cases of Bowen's disease), and condylomata acuminata of the external genitalia (33 cases). The patients were of Polish or French origin.

Fig. 2 Physical map of HPV33 DNA. The unique *Bgl*II site has been taken as the origin of the map. The distances from the origin are given in percentages of the HPV33 genome. Endonucleases that do not cleave the HPV33 DNA are: *Bam*HI, *Cl*aI, *Eco*RI, *Pvu*I, *Sac*I, *Sal*I and *Xho*I.



HPV33 DNA was detected in two cases of severe cervical dysplasias, in one additional case of invasive cervical carcinoma, in one case of bowenoid papulosis, and in three cases of Bowen's disease. The *Pst*I cleavage patterns of the DNA samples were similar to that of the prototype HPV33 DNA (Fig. 1B, lanes *g*, *k*), except for the DNA obtained from a biopsy of vulvar Bowen's disease, which yielded two fragments with abnormal mobilities (Fig. 1B, lane *o*).

Several bands, migrating as form I, II and III DNA molecules or exhibiting lower mobilities, were observed in five untreated DNA preparations (Fig. 1B, lanes *d*, *h*). The migration of these bands was not modified by treatment with a mixture of two 'no-cut' enzymes for HPV33 DNA (Fig. 1B, lanes *e*, *i*), and DNA molecules of one genome length were generated by treatment with a one-cut enzyme (Fig. 1B, lanes *f*, *j*). This result indicates that the slow-migrating species, predominant in a case of bowenoid papulosis and in the cancer from which HPV33 DNA was cloned, correspond to free oligomers (Fig. 1B, lane *d*). In contrast, in the preparation yielding an abnormal *Pst*I cleavage pattern, the viral DNA sequences were found exclusively associated with the bulk of the cellular DNA (Fig. 1B, lane *l*). A single 11-kb band was observed after treatment with a mixture of two no-cut enzymes (Fig. 1B, lane *m*), and two fragments longer than 8 kb each were detected after treatment with a one-cut enzyme (Fig. 1B, lane *n*), indicating that the HPV33 DNA sequences were integrated in a single site of the host-cell genome, with a 3-5-fold amplification of the integrated viral genome and of the flanking cellular sequences, as deduced from the intensity of the bands. After treatment with *Hind*III and *Pvu*II, the 2-kb *Hind*III fragment localized in a clockwise direction between 3.8 and 29.8 map units (MU) (Fig. 2), and the 3.9-kb *Pvu*II fragment localized between 73.5 and 22.5 MU (Fig. 2) were not detected (Fig. 1B, lanes *p*-*s*). This result indicates that integration interrupted the viral genome between 3.8 and 22.5 MU (Figs 2, 3C). Only one virus/cell DNA junction was detected after cleavage with *Hind*III (Fig. 1B, lane *q*), suggesting that the integration site was close to the *Hind*III site located at 3.8 MU in a region corresponding to the E2 ORF (Fig. 3C).

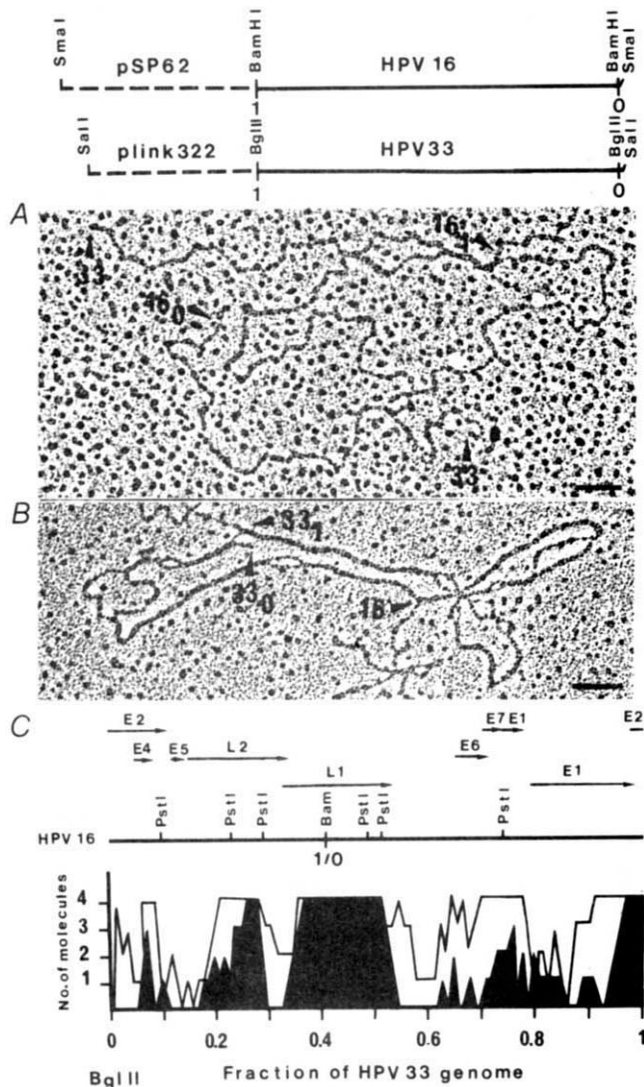


Fig. 3 Partial homology maps of HPV33 and HPV16 DNAs, as determined by heteroduplex analysis. pSP62/HPV16 (ref. 20) and plink322/HPV33 recombinant plasmids were cut at their *Sma*I and *Sal*I restriction sites located on the plasmids at 6 and 73 bp, respectively, from the *Bam*HI and *Bgl*II insertion sites^{9,34}. The physical maps of the linearized recombinant plasmids are shown at the top. The *Bam*HI insertion site has been taken as the origin of the HPV16 physical map¹⁹, and the 0→1 orientation corresponds to the 5'→3' direction of the HPV16 nucleotide sequence²⁶. **A**, Excised HPV33 DNA and *Sma*I-linearized pSP62/HPV16 recombinant plasmid were annealed in the presence of 30% formamide and spread in the presence of 60% formamide (T_m -20 °C)^{23,24}. Inspection and measurement of the heteroduplex molecules allowed the pSP62 plasmid sequences to be identified and the orientation of the HPV16 DNA to be determined. The plasmid-HPV16 DNA junctions and the extremities of the HPV33 DNA are shown. **B**, *Sma*I-linearized pSP62/HPV16 and *Sal*I-linearized plink322/HPV33 recombinant plasmids were annealed and spread in the presence of 30% formamide (T_m -39 °C). The difference in size between the pSP62 (4,250 bp)³⁴ and plink322 (3,800 bp)⁹ plasmids allowed us to identify the plasmid sequences, and to deduce the orientation of the HPV33 genome. The plasmid-HPV DNA junctions are shown. **C**, Partial homology maps were obtained by analysis of the heteroduplex molecules formed between *Sma*I-linearized pSP62/HPV16 and *Sal*I-linearized plink322/HPV33 recombinant plasmids, or between linearized pSP62/HPV16 plasmid and excised HPV33 DNA. The lengths of the single-stranded and double-stranded regions of HPV33/HPV16 heteroduplex molecules were measured using a digital length calculator (Numonics) connected to a Nova 3D computer (Data General). The histograms represent the numbers of double-stranded 0.01-genome-length segments observed in heteroduplex molecules formed in either 30% formamide (T_m -39 °C) (□) or 50% formamide (T_m -26 °C) (■), as a function of their position on the physical map of HPV33 DNA. The alignment of the HPV33 and HPV16 DNA maps was deduced from the oriented partial homology maps. A simplified physical map of HPV16 DNA¹⁹ and the arrangement of the open reading frames in the HPV16 genome²⁶ are shown above the histograms. Scale bar in **A**, **B** represents 0.1 μ m.

HPV33 constitutes a novel HPV type that is evolutionarily related to HPV16 and has biological properties similar to those of HPV16 and HPV18. Its prevalence seems low compared with that of HPV16, which, in our series of experiments, was detected in 43% and 63% of the intra-epithelial neoplasias and invasive carcinomas of the uterine cervix, respectively, and in 65% of the intra-epithelial neoplasias of the external genitalia. HPV18 was found in only 5% and 10%, respectively, of the intra-epithelial neoplasias and invasive carcinomas of the uterine cervix.

The available data on cervical carcinomas and derived cell lines indicate that HPV16 and HPV18 DNAs are integrated in the cell genomes^{4,19,27,28} and that the insertion site is usually located in a region of the viral DNA corresponding to the E1 or E2 ORFs^{27,28}. The Shope papillomavirus and the HPV5 genomes are usually detected as free monomers or oligomers in skin carcinomas of domestic rabbits²⁹ and patients suffering from epidermodysplasia verruciformis³⁰, respectively. In addition to integrated viral genomes, the presence of free HPV16 oligomers has also been reported in some genital cancers¹⁹. Here, HPV33 genomes were detected only as free monomers or oligomers in two cervical cancers, but evidence for the integration of HPV33 DNA sequences in the E2 ORF region of the viral genome was obtained in one case of vulvar carcinoma *in situ* (Bowen's disease).

Because of its properties, HPV33 should be considered as an additional type of genital HPV that has oncogenic potential.

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A mutated polyoma virus enhancer which is active in undifferentiated embryonal carcinoma cells is not repressed by adenovirus-2 E1A products

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Enhancers stimulate transcription of several eukaryotic genes (for review see ref. 1). While some enhancers, like that of simian virus 40 (SV40), are active in a wide range of cell types, others are more cell-specific¹. For example, the polyoma virus (Py) enhancer is not active in undifferentiated embryonal carcinoma (EC) cells, such as F9 cells, while it is active in differentiated cells^{2,3}. In contrast, the SV40 enhancer is active in both undifferentiated and differentiated EC cells⁴. One possible explanation for this difference is that the two viral enhancers interact with different positively or negatively acting transcription factors, a notion supported by *in vitro* experiments showing that the Py enhancer interacts with proteins that do not bind to the SV40 enhancer^{5,6}. Some viral^{7,8} and cellular⁹ enhancers, including the Py and SV40 enhancers, can be negatively regulated by the products of the E1A transcription unit of adenovirus-2. As it has been postulated that undifferentiated F9 cells contain an E1A-like activity¹⁰, it is possible that the latter is responsible for the lack of activity of the Py enhancer in these cells. We show here that the E1A products do not repress a point mutant of the Py enhancer (Py ECF9.1; ref. 11 and references therein) that is active in undifferentiated F9 cells³. This result is consistent with the idea that undifferentiated F9 cells contain a cellular repressor that blocks the Py enhancer and that this repressor has the same target sequence as the E1A proteins.

The following recombinants were constructed as described in Fig. 1. pG2 contains the entire rabbit β -globin gene cloned in pBR322; pG2B, pG2Py and pG2Py* were derived from pG2 by inserting the following upstream from the β -globin gene promoter: the SV40 enhancer containing a single 72-base pair (bp) sequence (pG2B), the Py wild-type enhancer (pG2Py) and its mutated counterpart (pG2Py*) derived from the Py mutant Py ECF9.1 (ref. 11). In recombinant pE2G the β -globin gene promoter is replaced by the adenovirus-2 (Ad-2) E2A promoter¹². The transcriptional activity of these recombinants was analysed in two mouse L-cell-derived cell lines, L-CMI and L-CMIE1A¹³. The latter cell line, which was obtained by stable integration of the Ad-2 E1A transcription unit in the genome of L-CMI cells, constitutively expresses the E1A gene at a low level¹³. Run-on

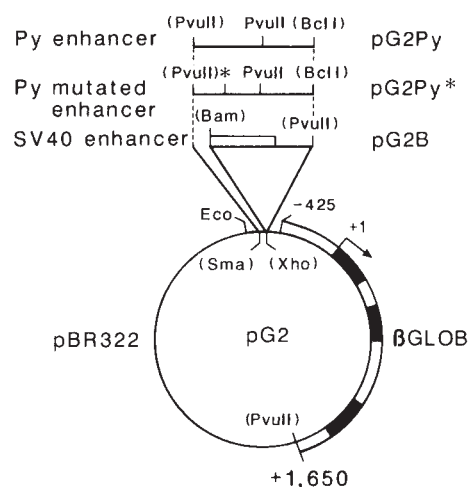


Fig. 1 Structure of the recombinants pG2, pG2B, pG2Py and pG2Py*. pG2 contains the rabbit β -globin gene from position -425 to position +1,650 (with respect to the β -globin cap site) inserted between the *EcoRI* and the *PvuII* sites¹⁷ of a pBR322 derivative carrying a polylinker containing a *XhoI* and a *SmaI* site in its *EcoRI* site. pG2B, pG2Py and pG2Py* were constructed by inserting the following fragments in the polylinker of pG2: pG2B, the *BamHI*(179)-*PvuII*(270) SV40 enhancer fragment (containing a single 72-bp sequence, boxed in the figure) excised from recombinant pA0¹⁸; pG2Py, the *PvuII*(5,262)-*BclI*(5,021) Py enhancer fragment¹⁹; pG2Py*, the *PvuII*-*BclI* Py enhancer fragment isolated from the Py ECF9.1 mutant^{3,11} which contains the transition A $\cdot$ T $\rightarrow$ G $\cdot$ C at position 5,233. Solid boxes in the β -globin gene sequence represent exons. None of the recombinants is drawn to scale. Restriction sites in parentheses were destroyed during the construction of the recombinants.

transcription experiments performed with nuclei isolated from L-CMI and L-CMIE1A cells (Fig. 2a) showed that the E1A gene was transcribed in the L-CMIE1A cells, and that the level of transcription of several cellular genes (actin (Act), glyceraldehyde phosphate dehydrogenase (GAPDH) and triose phosphate isomerase (TPI)) and of the VL30 retrovirus-like repetitive sequences, was not affected by the presence of the E1A products in this cell line (compare L-CMIE1A with L-CMI in Fig. 2a).

L-CMI and L-CMIE1A cells were transfected with recombinants pG2, pG2Py and pE2G, and the concentration of β -globin messenger RNA was analysed by quantitative *S*₁ nuclease mapping (Fig. 2b). In keeping with the known effect of the E1A products on transcription from the Ad-2 E2A gene (ref. 12 and references therein), the level of RNA initiated at the E2A promoter of recombinant pE2G was higher in L-CMIE1A than in L-CMI cells (Fig. 2b, lanes 2 and 5) under conditions where transcription from the β -globin gene promoter alone in recombinant pG2 was identical in the two cell lines (lanes 1 and 4). In contrast, the level of RNA transcribed from the globin gene promoter under the control of the Py enhancer (pG2Py) was weaker (fivefold weaker on average in three independent experiments) in L-CMIE1A than in L-CMI cells (Fig. 2b, lanes 3 and 6). Thus, as expected from our previous results⁷, the presence of the E1A products in the L-CMIE1A cell line results in an approximately fivefold repression of the activity of the Py enhancer. This moderate repression probably results from the low amounts of E1A products in these cells¹³. In our previous experiments⁷, a similar repression was obtained when cells were transfected with very low amounts of an E1A gene-containing recombinant. Therefore, it seems that the L-CMIE1A cells are suitable for studies of the repression of the Py enhancer by the E1A products⁷.

Recombinants pG2, pG2B, pG2Py and pG2Py* were transfected into the following cell lines: HeLa, L-CMI, L-CMIE1A and undifferentiated F9 cells. Transcription from the β -globin gene

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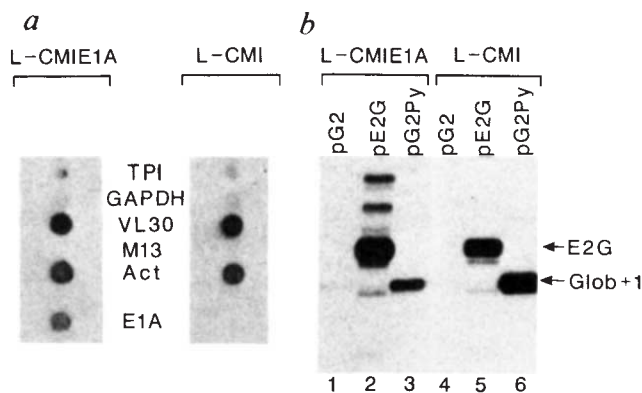


Fig. 2 Activity of the polyoma virus enhancer is inhibited in a cell line (L-CMIE1A) expressing constitutively the Ad-2 E1A products. The L-CMIE1A cell line was obtained by co-transfecting the L-CMI cell line (a mouse L-cell derivative; ref. 13) with the recombinant pE1ASV (which contains the entire Ad-2 E1A gene⁷) and the recombinant pY3 carrying a gene that confers hygromycin resistance²⁰. *a*, Nuclear run-on transcription analysis performed as described in refs 9 and 12 on $\sim 10^7$ nuclei from L-CMIE1A and L-CMI cells. One μ g of each of the following DNAs was spotted onto nitrocellulose filters: TPI, a rat triose phosphate isomerase complementary DNA fragment²¹; GAPDH, a rat glyceraldehyde phosphate dehydrogenase cDNA fragment²¹; VL30, a genomic fragment of the VL30 retrovirus-like mouse repetitive sequence²¹; Act, a rat actin cDNA fragment²¹; M13, Mp7 DNA; E1A, ME1ASV DNA⁷ containing the entire Ad-2 gene cloned in M13. *b*, S₁ nuclease analysis of 10 μ g of cytoplasmic RNA from L-CMIE1A or L-CMI cells transfected with 2 μ g of the recombinants pG2, pE2G or pG2Py using the calcium phosphate technique²². pG2 and pG2Py are described in Fig. 1 legend. pE2G¹² contains the Ad-2 E2A early promoter linked to a promoterless rabbit β -globin gene. RNA was extracted and analysed by quantitative S₁ nuclease mapping as described in ref. 22, using a β -globin probe which yields a protected fragment of 134 nucleotides (labelled Glob + 1) with recombinants pG2 and pG2Py, and a protected fragment of 143 nucleotides (E2G) with recombinant pE2G. Similar results were obtained in several transfection experiments in which different plasmid preparations were used.

promoter (pG2) was stimulated to a similar extent by the SV40 (pG2B), polyoma virus (pG2Py) and mutated polyoma virus (pG2Py*) enhancers in HeLa (Fig. 3, lanes 1–4) and L-CMI (Fig. 3, lanes 5–8) cells. (In agreement with a previous report³, the mutated Py enhancer was slightly but reproducibly more active than the wild-type enhancer in the mouse L-CMI cell line.) In agreement with the results shown in Fig. 2, the wild-type Py enhancer (pG2Py) was less efficient at stimulating the globin gene promoter in the E1A-transformed L-CMIE1A cells than in the parental L-CMI cells or in HeLa cells (Fig. 3, compare within each panel the lane labelled pG2Py with lanes pG2B and pG2). In contrast, the mutated Py enhancer efficiently stimulated the β -globin gene promoter in all three cell lines (Fig. 3, compare within each panel lanes labelled pG2Py* with lanes pG2B and pG2). Thus, expression of the E1A proteins does not lead to a decrease in the activity of the mutated Py enhancer in L-CMIE1A cells. The only difference in transcription which could be detected between the L-CMI and L-CMIE1A cells was the constitutive expression of the E1A products at a low level (see Fig. 2a). In addition, the presence of these products led, as could be expected, to the stimulation of the E2A promoter and the repression of the wild-type Py enhancer (see Fig. 2b). Therefore we conclude that, in contrast to the wild-type Py enhancer, the mutated Py enhancer is resistant to repression by the E1A products, although at present we cannot exclude the possibility that some as yet unknown events which might have occurred during the establishment of the L-CMIE1A cell line could in fact be responsible for the apparent lack of inhibition of the mutated Py enhancer in L-CMIE1A cells.

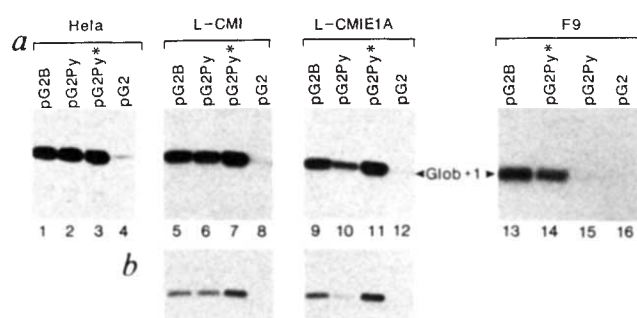


Fig. 3 The point-mutated Py enhancer is not inhibited by the E1A products. *a*, S₁ nuclease analysis of 10 μ g of cytoplasmic RNA extracted from HeLa, L-CMI, L-CMIE1A and F9 undifferentiated embryonal carcinoma cells, after transfection (using the calcium phosphate technique) of 2 μ g of pG2B, pG2Py, pG2Py* or pG2 DNA (see Fig. 1). RNA was extracted and analysed with a β -globin gene probe as described in Fig. 2 legend. *b*, A shorter exposure of the autoradiograms corresponding to lanes 5–12 in *a*. The results shown are representative of a series of transfection experiments which were carried out with independent preparations of plasmids.

Note that in the present experiments the activity of the SV40 enhancer was not significantly modified by the E1A products, whereas we have shown previously that it can be repressed when co-transfected with E1A gene-containing recombinants⁷. This apparent discrepancy probably results from the low amount of E1A products made in L-CMIE1A cells¹³, as our previous transfection experiments have shown that the Py enhancer required less E1A products to be repressed than the SV40 enhancer. That the lack of repression of the SV40 enhancer in L-CMIE1A cells was related to a low level of expression of the E1A products is supported by the observation that the wild-type Py enhancer became almost as active as the mutated one when increasing amounts of pG2Py and pG2Py* were transfected (data not shown). As expected from previous reports^{3,11} when the same recombinants (pG2, pG2B, pG2Py and pG2Py*) were transfected in undifferentiated F9 cells, the Py wild-type enhancer was almost inactive (Fig. 3a, lane 15), while the SV40 (lane 13) and the mutated Py (lane 14) enhancers were both similarly active.

As proposed previously^{3,11}, the generation of an additional enhancer motif could represent the mechanism by which the enhancer of the Py mutant Py ECF9.1 becomes active in undifferentiated EC cells as a consequence of a single point mutation. Recent *in vivo* competition experiments which have shown that both the Moloney sarcoma virus¹⁴ and polyoma virus¹⁵ enhancers are repressed by a *trans*-acting factor(s) (repressor) in undifferentiated EC cells, have raised the possibility that the activity of the mutated Py enhancer could also result from the removal of a negative regulation. Our results, which indicate that the mutation present in the mutated Py ECF9.1 enhancer overcomes the repression by the E1A products, support this possibility and also suggest that the repressor present in undifferentiated F9 cells and the E1A proteins have the same DNA target. That undifferentiated F9 cells may contain an E1A-like activity has in fact been suggested previously to account for their ability to support the growth of the Ad-5 E1A-deleted mutant dl312 (ref. 10). The physiological role of the E1A-like activity in undifferentiated EC cells is unknown; one proposal is that it could be involved in some way in the control of the cell cycle¹⁶. Our previous⁷ and present results suggest that this E1A-like activity may also repress some cellular enhancers whose activity is required only after differentiation has started.

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Phylogenetic distribution of *Antennapedia*-like homoeo boxes

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The homoeo box is a conserved protein-coding DNA sequence present in several genes in arthropods, annelids and vertebrates¹⁻⁴. Two distinct classes of homoeo box have been identified—the *Antennapedia* (*Antp*) class^{1,5} and the *engrailed* class⁶⁻⁸. In insects, both classes are found in genes involved in regulating development. We have therefore investigated the phylogenetic distribution of *Antp*-like homoeo boxes to explore the possibility of a link between their presence and developmental strategy^{1,9-11}. The distribution data also give important insight into the origin of homoeo boxes and the phylogeny of the animal kingdom.

The organisms used in this study are from a range of organizational grades and were chosen to extend the data previously reported. They were one chordate (mouse *Mus musculus*), two echinoderms (starfish *Asterias forbesi*; sea urchin *Paracentrotus lividus*), one brachiopod (*Terebratulina* sp.), two molluscs (snail *Biomphalaria glabrata*; sea hare *Aplysia californica*), two nematodes (*Nippostrongylus brasiliensis*; *Strongylus vulgaris*), one nemertean (*Lineus longissimus*), two platyhelminthes (tapeworm *Echinococcus granulosus*; digenean fluke *Schistosoma mansoni*), fission yeast (*Schizosaccharomyces pombe*) and slime mould (*Dictyostelium discoideum*).

To test for the presence of homoeo-box sequences, Southern blots were prepared from *Eco*RI-digested genomic DNA from these 13 organisms, then hybridized with two ³²P-labelled homoeo-box probes (A and M) under conditions of reduced stringency¹. Probe A was derived from a *Drosophila Antp* complementary DNA p903 (refs 1, 12) by W. D. Richardson, and contains the entire 180-base-pair (bp) homoeo box plus only 40 bp of flanking sequence. Probe M is a 600-bp *Eco*RI-*Pvu*II fragment from the mouse genomic clone H24.1 (ref. 13), and

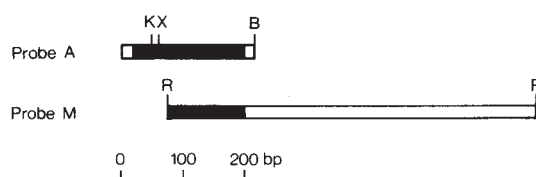


Fig. 1 The two homoeo-box probes shown schematically. Direction of transcription in each gene is from left to right. The shaded region represents the conserved homoeo box. B, *Bam*HI; K, *Kpn*I; P, *Pvu*II; R, *Eco*RI; X, *Xba*I.

contains 129 bp of a mouse *Antp*-like homoeo box. (The probes are shown schematically in Fig. 1.) The only sequence homology between the probes is the homoeo box, which is 84% identical over the shared 3' portion. Hence, if the same bands on a genomic DNA blot are detected with both probes, it is very likely that the signals are derived from homoeo-box sequences. Bands detected by one probe only could represent divergent homoeo boxes, but in this case their identification is less certain. It has been suggested that under conditions of reduced stringency spurious hybridization can occur to ribosomal DNA repeats¹⁴. Therefore, after the two homoeo-box hybridizations, each blot was probed with a nick-translated *Xenopus laevis* ribosomal DNA probe (probe R) at reduced stringency. The results of the three hybridizations to each species are shown in Fig. 2.

Five of the species tested (mouse, starfish, sea urchin and both molluscs) clearly hybridized to both homoeo-box probes, with three to eight bands in common between the two probes. The hybridization patterns did not correspond to the ribosomal DNA bands, and in every case bands were detected with probes A and M which were not seen with the ribosomal probe. These species were therefore considered positive for the presence of homoeo-box sequences. The conclusion that homoeo boxes are present in Mollusca conflicts with a recent report by McGinnis¹⁴, in which hybridizing sequences were not detected in two different mollusc species from those used here.

In the nemertean, each probe hybridized to only two bands, with one band position in common (Fig. 2). A similar result was obtained for the brachiopod, where three bands were detected by probe A, with one band position in common with probe M. It is possible that in each case the shared band represents a true homoeo-box gene. If this is the case, then either these organisms have only one homoeo box, or any others which may be present have diverged beyond the limit of detection.

In the tapeworm, clear hybridization to multiple bands was observed with probe A. As this probe consists almost entirely of homoeo-box sequences (180 bp/220 bp), this result suggests that homoeo boxes are present. However, none of the bands hybridized with probe M (Fig. 2). One possibility is that the hybridization seen with probe A results from chance sequence similarity between tapeworm repeated sequences and the short (40 bp) non-homoeo-box sequences in probe A. Alternatively, the bands could represent true homoeo boxes which have diverged sufficiently to be undetected by probe M while retaining sequence homology to the *Antp* homoeo box. In the nemertean, brachiopod and tapeworm, the hybridizing bands did not correspond to ribosomal DNA bands (Fig. 2), and in no case were bands of higher DNA concentration revealed by ethidium bromide staining of the gel.

The remaining five species tested (digenean fluke, both nematodes, yeast and slime mould) were consistently negative with both probes. It is important that in all these cases, however, the blots showed clear hybridization to the ribosomal probe (Fig. 2), indicating that the DNA transferred efficiently and was undegraded. These organisms were therefore considered to lack genes containing *Antp*-like homoeo boxes.

To summarize the results from the present study and that of

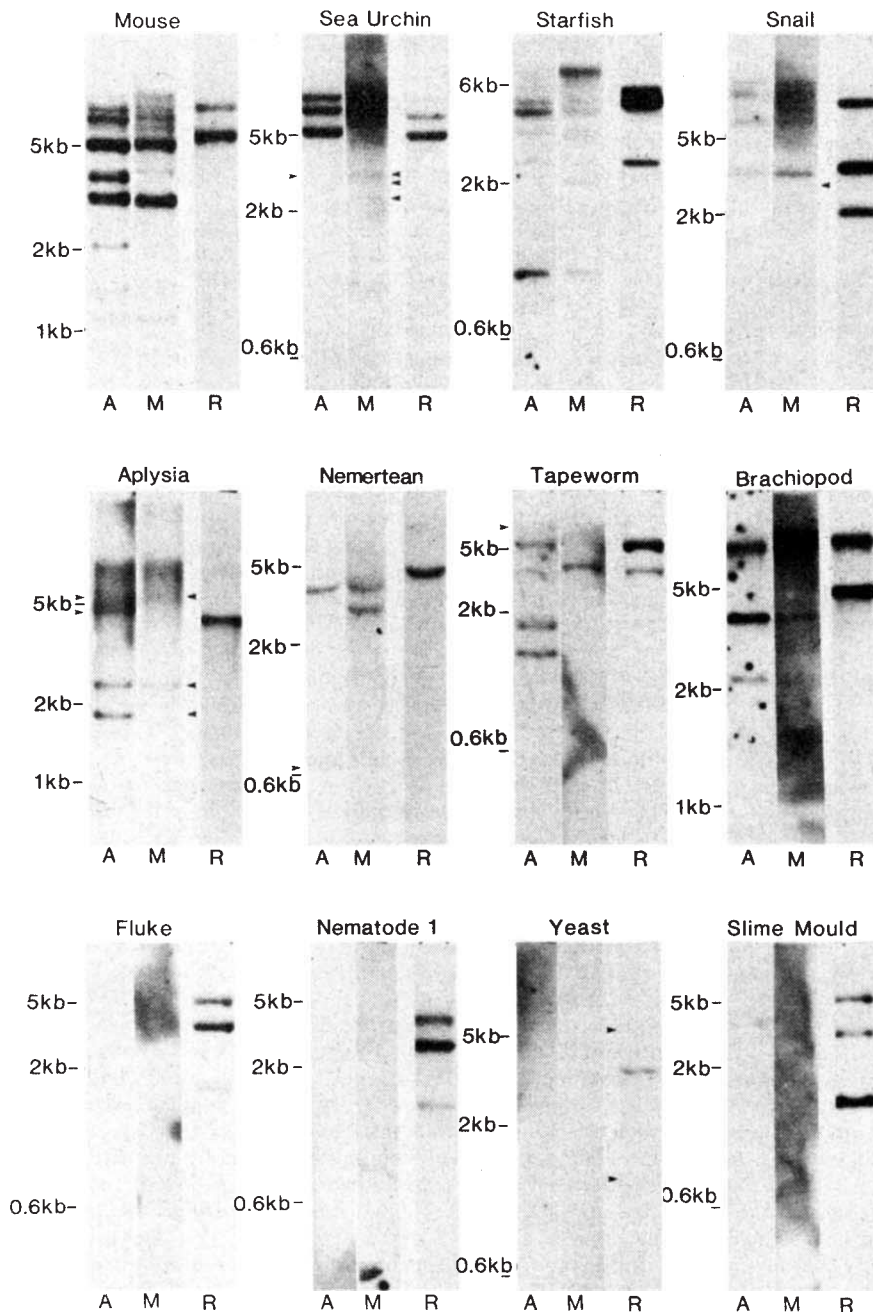


Fig. 2 Autoradiographs showing hybridization of homoeo-box probes A and M and ribosomal probe R, to genomic Southern blots. Nematode 1, *N. brasiliensis*. Similar results were seen with the nematode *S. vulgaris*. Arrowheads indicate the positions of faint bands.

Methods. Genomic DNA was prepared from *Lineus* (University Marine Biological Station, Millport, UK) and *Terebratulina* by incubating fresh minced tissue overnight in SDS/proteinase K, followed by phenol/chloroform extraction and ethanol precipitation²². *Terebratulina* DNA was further purified by two bandings on CsCl gradients²³ followed by dialysis, phenol/chloroform extraction and ethanol precipitation. The other DNA samples were donated. Genomic DNA from each organism was digested to completion with *EcoRI*, and Southern blots performed as described by Maniatis *et al.*²⁴. Each lane contained 10 µg of DNA, except for *Asterias*, *Terebratulina*, *Nippostrongylus* and *Strongylus* (5 µg), yeast (1 µg) and *Dictyostelium* (0.5 µg). Every blot performed included a mouse DNA lane as a positive control. Cloned DNA fragments A and M were isolated from agarose gels, and labelled by nick-translation to an average specific activity of 3×10^8 d.p.m. µg⁻¹. Test hybridizations demonstrated that the level of contamination of the isolated inserts by plasmid sequences was negligible. For the control ribosomal hybridization the entire recombinant plasmid was nick-translated. This clone contains sequences from both 18S and 28S ribosomal DNA. Filters were prehybridized overnight in 43% deionized formamide, 5×SSC, 0.1% bovine serum albumin, 0.1% Ficoll 400, 0.1% polyvinylpyrrolidone, 0.1% SDS, 50 mM sodium phosphate buffer pH 7, 250 µg ml⁻¹ yeast total RNA at 37°C¹. This solution was removed and the filters were hybridized for 40 h at 37°C in the same buffer containing 2×10^6 d.p.m. of denatured probe (A, M or R) per ml of buffer. Filters were washed to 2×SSC/0.1% SDS at 50°C before being exposed to Kodak XAR-5 film at -70°C for 2-6 days (for probes A or M) with two intensifying screens. Before rehybridization, filters were stripped in 50 mM NaOH for 30 min at room temperature. Second and subsequent homoeo-box hybridizations required 2-10 days' exposure. The ribosomal hybridizations required 3-30 h exposure.

McGinnis *et al.*¹, *Antp*-like homoeo boxes are present in the genomes of arthropods, annelids, molluscs, chordates and echinoderms. They have not been detected in nematodes or flukes, and are either poorly conserved or present in low numbers in brachiopods, nemerteans and tapeworms. This phylogenetic distribution is depicted in Fig. 3.

It is clear that homoeo-box sequences are present in a wide range of animals possessing very different developmental strategies. These include the segmented arthropods, annelids and vertebrates, as well as the primitively unsegmented echinoderms and molluscs. Hence, the presence of *Antp*-like homoeo boxes seems not to be closely linked with segmentation as the earliest distribution patterns suggested^{1,9,11}. Furthermore, the phylogenetic distribution shows no obvious correlation with any developmental strategy. It is, however, possible that during evolution the homoeo box has been recruited for a variety of roles in different evolutionary lineages. The suggestion^{15,16} and

recent evidence¹⁷ that homoeo boxes encode DNA-binding domains of proteins implies that even if homoeo-box genes have different functions in distantly related organisms, it is likely that all such functions involve interactions with DNA. It is possible that many classes of regulatory genes, not only those controlling morphogenesis, would require this property.

The data presented here have implications for invertebrate phylogeny if the following assumptions are made. First, that the homoeo boxes in different phyla are truly homologous and not the result of convergent evolution. If this assumption is correct, *Antp*-like homoeo boxes must have arisen before the divergence of arthropods, molluscs and chordates. The data presented here cannot resolve the nature of their common ancestor, but it can help evaluate theories proposed on other evidence. Second, that the negative results seen are not caused by the secondary loss or divergence of the sequence. If this is true, then the arthropod, mollusc and chordate lineages must have separated after the

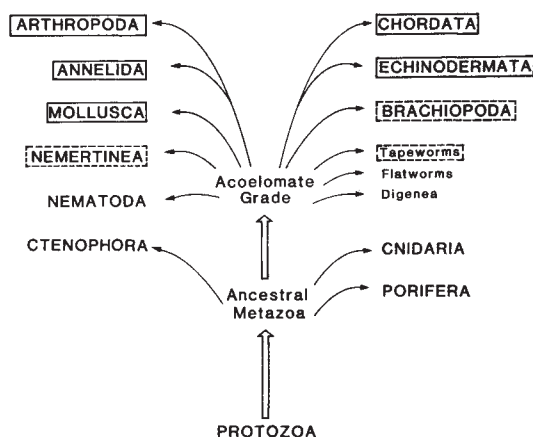


Fig. 3 A possible phylogeny of the animal kingdom. Sequences hybridizing to *Antp*-like homoeo boxes have so far been detected in the phyla enclosed by boxes. Organisms enclosed by dotted lines have fewer, or more divergent, homoeo-box-like sequences. Alternative phylogenies to the one shown are possible (see text). A number of smaller phyla have been omitted from this diagram.

divergence of the lineages giving rise to the nematodes and at least some platyhelminthes. This conclusion is consistent both with a phylogeny in which all coelomate phyla (including arthropods, annelids, molluscs, chordates, echinoderms and brachiopods) are derived from a common coelomate ancestor, and with phylogenies in which some or all of these phyla are derived independently from the acoelomate grade of organization^{18,19} (Fig. 3). It is, however, inconsistent with phylogenies which place this common ancestor much earlier, for example among the earliest metazoa or the protozoa^{20,21}.

Further insight into both invertebrate phylogeny and the evolution of the functions of homoeo-box gene products could be obtained by extending these experiments to screening with *engrailed* sequences, and with protein-coding sequences that flank homoeo boxes.

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Peptide chemotaxis in *E. coli* involves the Tap signal transducer and the dipeptide permease

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Bacterial chemotaxis provides a simple model system for the more complex sensory responses of multicellular eukaryotic organisms¹. In *Escherichia coli*, methylation and demethylation of four related membrane proteins, the methyl-accepting chemotaxis proteins (or MCPs), is central to chemotactic sensing and signal transduction². Three of these proteins, Tar, Tsr and Trg, have been assigned specific roles in chemotaxis. However, the role of the fourth MCP, Tap, has remained obscure³. We demonstrate here that Tap functions as a conventional signal transducer, enabling the cell to respond chemotactically to dipeptides. This provides the first evidence of specific bacterial chemotaxis towards peptides. Peptide taxis requires the function of a periplasmic component of the dipeptide permease. This protein represents the first example of a periplasmic chemoreceptor that does not have a sugar substrate.

In order to evoke a chemotactic response, amino-acid attractants bind directly to one or other of the signal transducers^{2,4}. On the other hand, sugars bind first to high-affinity binding proteins located in the periplasm^{2,5}; binding induces a conformational change in the protein⁶ which allows the substrate-binding protein complex to interact with the appropriate transducer. An unambiguous role in chemotaxis has been assigned to three of the four signal transducers. Tsr (taxis to serine and from repellents) mediates taxis towards the potent attractant L-serine (and related amino acids)⁷ and taxis away from certain repellents⁸. Tar (taxis to aspartate and from repellents) mediates taxis towards the strong attractants L-aspartic acid and maltose⁹ and taxis away from Ni²⁺ and Co²⁺ (ref. 8). Trg (taxis to ribose and galactose) is required for chemotaxis towards galactose and ribose¹⁰. Between them, the three transducing proteins can account for the bacterial responses to all known attractants and repellents that require a methyl-accepting signal transducer. The gene encoding the fourth signal transducer, Tap (taxis associated protein), has been characterized in some detail. The *tap* gene is immediately distal to *tar* in the *meche* operon¹¹, and sequencing¹² reveals that it has considerable homology with the *tsr* and *tar* genes¹³. However, no function has yet been assigned to this protein; deletion of *tap* has no significant effect on chemotaxis towards any known attractant or away from repellent³.

Small peptides play an important part in bacterial nutrition and are frequently abundant in the natural environment of enteric bacteria. For this reason, and because of the recent identification of a periplasmic oligopeptide-binding protein, which could potentially serve as a chemoreceptor^{14,15}, it seemed possible that *E. coli* might exhibit chemotaxis towards defined peptides and that peptide taxis might provide a role for Tap.

Three genetically distinct peptide transport systems function in *E. coli* and *Salmonella typhimurium*^{16–18}. The most completely characterized system, the oligopeptide permease (Opp), is encoded by an operon of five genes, all of which have been sequenced^{19–21}. The first of these genes, *oppA*, encodes a periplasmic oligopeptide-binding protein with an affinity for most

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Table 1 Comparison of various peptides as chemoattractants

Peptide	No. of cells in capillary			
	10 μ M	100 μ M	1 mM	10 mM
Dipeptides				
Gly-Gly	230	3,600	15,000	1,800
Gly-L-Leu	1,100	13,000	33,000	1,700
Gly-L-Pro	1,400	12,000	18,000	2,100
Gly-L-Val	400	11,000	10,000	1,200
L-Leu-Gly	0	8,700	29,000	5,400
L-Leu-L-Leu	1,200	2,600	2,800	400
L-Leu-L-Pro	1,300	12,000	7,000	3,000
L-Pro-Gly	660	9,300	23,000	8,200
L-Pro-L-Leu	1,500	12,600	4,400	1,400
L-Pro-L-Phe	700	8,700	2,800	500
L-Val-Gly	600	7,100	17,000	3,900
Dipeptides containing D-amino acids				
Gly-D-Leu	0	350	850	5,000
D-Leu-Gly	350	250	500	200
Tripeptides				
Gly-Gly-Gly	0	100	400	400
Gly-Gly-L-Ile	0	0	450	7,200
L-Leu-Gly-Gly	200	100	600	2,000
L-Leu-L-Leu-L-Leu	600	0	2,000	Insoluble
L-Pro-Gly-Gly	0	0	70	250
L-Val-Gly-Gly	0	0	100	1,200

The number of cells accumulating in capillaries containing the indicated concentrations of peptides was determined as described in Table 2 legend.

peptides containing up to five amino-acid residues and which shows little selectivity for the specific amino-acid composition of the peptide^{14,15,18}. The second peptide transport system, the tripeptide permease (Tpp), has a marked selectivity for hydrophobic tripeptides, although this specificity is by no means absolute²². Expression of *tppB*, which encodes the structural component(s) of this transport system, is induced by anaerobiosis or by leucine and, in addition, requires a functional OmpR protein^{22,23}. The third and most poorly characterized of the three transport systems is the dipeptide permease (Dpp). Dpp has high affinity for most dipeptides although this specificity is not absolute and some tripeptides can also be transported (refs 16, 18 and M. M. Gibson and C.F.H., in preparation). Mutations causing deficiency of Dpp function have been mapped to 80 min on the *S. typhimurium* chromosome (ref. 16 and M. M. Gibson and C.F.H., in preparation).

The motile *E. coli* strain RP437 (ref. 24) was tested for chemotaxis towards a variety of di- and tripeptides using the capillary assay²⁵. Many dipeptides were found to be good attractants although, to our surprise, tripeptides were generally poor attractants (Table 1). Several lines of evidence show that this response is towards the peptide rather than to contaminating amino acids or to amino acids released following uptake and cleavage of the peptide: (1) many peptides were attractants although they consisted entirely of amino acids such as isoleucine, leucine, phenylalanine, proline and valine, which individually cannot serve as attractants for *E. coli*⁷; (2) responses to dipeptides were always significantly greater than responses to their constituent amino acids at equivalent concentrations (data not shown); (3) mutations in *tar* and *tsr* had little effect on peptide taxis whereas taxis was inhibited in strains harbouring peptide transport mutations (see below).

To examine the effectiveness of different peptides as attractants, taxis was assayed in a derivative of RP437 which contains the *tsr-7021* deletion²⁶. This strain showed the best response to dipeptides (see below). A further advantage of using this strain is that a *tsr* mutant cannot respond to glycine (ref. 7 and M.D.M., unpublished data), which could be present as a contaminant in the peptide samples. Every dipeptide examined that contained only L-amino acids or glycine was found to be an attractant in

capillary assays, with peak accumulations occurring at peptide concentrations of 100 μ M to 1 mM (Table 1). Peptides containing a D-amino acid were much poorer attractants than their L-amino acid counterparts. Tripeptides were generally found to be poor attractants, although some, such as glycylglycyl-L-isoleucine, could serve as reasonably good attractants at high concentration (10 mM). Thus, the peptide chemoreceptor can recognize a wide range of dipeptides made up of L-amino acids, but discriminates against tripeptides (and presumably larger peptides also) and against peptides containing D-amino-acid residues.

As the MCPs are involved in sensing most chemoattractants, it was important to ascertain which, if any, of these signal transducers is involved in dipeptide taxis. Therefore, we measured peptide taxis in strains which were isogenic except for defined deletions in one or more of the transducer genes. The results were unambiguous: all strains deleted for *tap* lacked peptide taxis while all strains containing an intact *tap* gene showed wild-type peptide taxis (provided either *tsr* or *tar* was also intact; strains lacking both *Tsr* and *Tar* are generally non-chemotactic, even when the *Trg* and *Tap* proteins are present at normal levels¹¹). Table 2 presents results obtained for the peptide L-prolylglycine; however, essentially identical results were obtained for all other peptides tested. Thus, the *Tap* transducer must have a central role in peptide taxis. Consistent with this notion is the finding that a motile derivative of *S. typhimurium* LT2 (ref. 27), which possesses all three peptide transport systems, totally lacks peptide taxis but still exhibits normal taxis towards serine and aspartate; *S. typhimurium* is known to lack the *tap* gene³.

To quantitate the chemotactic response, taxis to various peptides was measured in a flow chamber²⁸ using the tethered-cell method²⁹. The response time with saturating stimuli was 40–80 s, which is comparable to that observed for galactose and ribose³⁰. In contrast, under the same conditions maltose has a maximum response time of 2.5–4 min^{29,31}. This difference in response time has been attributed to the relative abundance of the *Trg* and *Tar* signal transducers^{2,30}. *Trg*, the transducer for ribose and galactose, is present in only a few hundred copies per cell whereas *Tar*, the transducer for maltose, is present in ~1,000 copies². Thus, one would predict that *Tap* is a minor transducer, about as abundant as *Trg*. Estimates of the number of *Tap* molecules present per cell indicate that this is indeed the case¹¹.

What is the chemotactic receptor for dipeptides? Chemo-effectors can either bind directly to the signal transducers or,

Table 2 Peptide chemotaxis in various transducer mutants

Strain	Intact transducers	Capillary content*	No. of cells in capillary
RP437 ²⁴ (<i>phoA8</i>)	<i>Tsr</i> , <i>Tar</i> , <i>Tap</i> , <i>Trg</i>	L-Serine	59,000
		L-Aspartate	105,000
		L-Prolylglycine	6,600
RP437 (<i>phoA8</i> Δ <i>tsr7021</i> [†] <i>thr</i> ⁺)	<i>Tar</i> , <i>Tap</i> , <i>Trg</i>	L-Serine	700
		L-Aspartate	84,000
		L-Prolylglycine	13,000
RP437 (<i>phoA8</i> Δ <i>tar-tap5201</i> [‡] <i>eda</i> ⁺)	<i>Tsr</i> , <i>Trg</i>	L-Serine	34,000
		L-Aspartate	100
		L-Prolylglycine	0
RP2361 (RP437 Δ <i>tar386-28</i>)	<i>Tsr</i> , <i>Tap</i> , <i>Trg</i>	L-Serine	55,000
		L-Aspartate	100
		L-Prolylglycine	3,500
RP3525 (RP437 Δ <i>tap365-48</i> <i>thr</i> ⁺ <i>leuB</i> ⁺ Δ [<i>argF-lac</i>]U169)	<i>Tsr</i> , <i>Tar</i> , <i>Trg</i>	L-Serine	70,000
		L-Aspartate	59,000
		L-Prolylglycine	100

Cells were grown and capillary tests²⁵ were performed as described previously²⁹. The accumulation (400–800 cells) in capillaries containing chemotaxis buffer alone was subtracted as background; values given are means for two capillaries. The *phoA8* marker, introduced for other experiments, had no effect on chemotaxis in strain RP437.

* 1 mM in each case.

[†] Ref. 26; [‡] ref. 11; § ref. 3.

alternatively, are first bound by a periplasmic binding protein which subsequently interacts with the transducer. The dipeptide receptor must have high affinity for its substrates as, in experiments with tethered cells, a maximal response was elicited by only 1 μ M of L-prolyl-L-leucine or L-leucyl-L-leucine. At least 10-fold higher concentrations of glycylglycine and L-prolylglycine were required to saturate the response, although at saturating concentrations of peptide the length of response was comparable for all four peptides. These differences reflect the relative peptide affinities determined in capillary assays (Table 1). The high affinity of the chemoreceptor for peptides, and the kinetics of peptide chemotaxis, suggest the involvement of a periplasmic peptide-binding protein.

In addition to the role of certain periplasmic binding proteins as chemoreceptors, these proteins also provide the initial receptor for their respective transport systems^{2,32,33}. Initially, the periplasmic oligopeptide-binding protein seemed an obvious candidate for a peptide chemoreceptor. However, the substrate specificity observed here for chemotaxis was rather different from the transport and binding specificity of OppA^{15,16,18}. A role for OppA was completely excluded by the finding, by immunoblotting, that RP437 is *opp*⁻ and lacks the OppA protein. An *opp*⁺ derivative of RP437 was constructed by phage P1 transduction and a functional OppA protein restored; rather than being increased, peptide chemotaxis was actually reduced in the *oppA*⁺ derivative. Presumably, OppA competes with the chemoreceptor for peptide binding.

It is not known whether transport via either of the other two peptide permeases, Tpp and Dpp, involves a periplasmic peptide-binding protein. Unfortunately, well-defined *tpp* and *dpp* mutants do not exist for *E. coli*. However, such derivatives have been constructed in *S. typhimurium*. It is clear that the substrate specificity for chemotaxis in *E. coli* is very similar to that for transport via Dpp in *S. typhimurium* (refs 16, 18 and M. M. Gibson and C.F.H., in preparation). Kinetic competition experiments have indicated a similar substrate specificity for Dpp in *E. coli*^{18,34}. In addition, the observation that glycylglycine transport in *E. coli* is sensitive to osmotic shock³⁵ indicates a role for a periplasmic peptide-binding protein in dipeptide transport. To identify such a protein, the periplasmic proteins from several independently isolated and well-characterized *dpp* mutants of *S. typhimurium* (M. M. Gibson and C.F.H., in preparation) were examined by SDS-polyacrylamide gel electrophoresis (Fig. 1b). About 20% of these mutants lacked a periplasmic protein with a relative molecular mass (M_r) of ~49,000 (49K). On transduction of these *dpp* mutations into a wild-type strain, 100% correlation was observed between loss of Dpp activity and loss of the 49K protein. It is interesting that this periplasmic dipeptide transport component is one of the most abundant periplasmic proteins (equivalent in amount to the fully induced maltose- or galactose-binding proteins) and is also considerably larger than all other known substrate-binding proteins except for OppA, which has a M_r of 58K^{14,15,21}.

Because *S. typhimurium* lacks the *tap* gene, and does not show peptide chemotaxis, we were unable to prove that this Dpp-associated periplasmic protein is required for chemotaxis. We therefore searched for an equivalent protein in *E. coli*. An abundant 49K periplasmic protein was present in the periplasm of *E. coli* strain RP437 (Fig. 1a). Significantly, the amount of this protein was at least 10-fold lower in a motile derivative of strain MC4100 (ref. 31), which has greatly reduced dipeptide transport and peptide taxis (results not shown). Using the toxic peptide bacilysin¹⁶, we isolated 24 spontaneous resistant mutants of *E. coli* strain in RP437 which, by analogy with *S. typhimurium* (ref. 16 and M. M. Gibson and C.F.H., in preparation), we anticipated to be Dpp⁻. These mutants were indeed found to be defective in dipeptide transport. Significantly, 15% of the presumed *dpp* mutants lacked the 49K periplasmic protein; no other periplasmic protein was altered in any of the mutants (Fig. 1a). Therefore, it seems that, like *S. typhimurium*, *E. coli* also

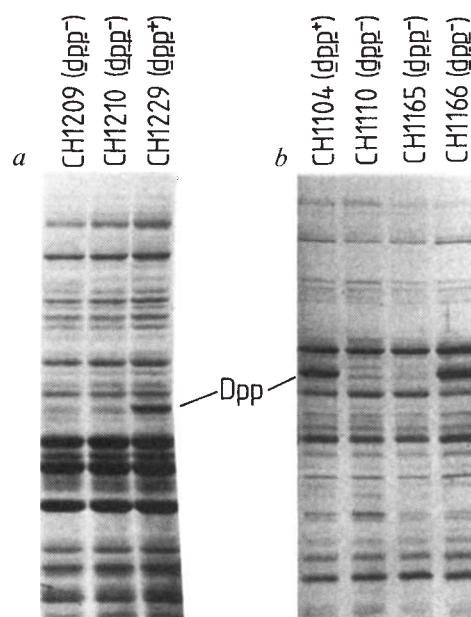


Fig. 1 SDS-polyacrylamide gel electrophoresis of periplasmic proteins from various *E. coli* (a) and *S. typhimurium* (b) strains. Periplasmic proteins were isolated by osmotic shock and separated on 9% SDS-polyacrylamide gels as described previously¹⁴. The Dpp periplasmic protein is indicated; its M_r was estimated to be 49K by comparison with markers of known M_r . The genotypes of the *E. coli* K-12 strains are as follows: CH1229, RP437 *opp*-466 *ompR*::Tn10; CH1209, CH1229 *dpp*-465; CH1210, CH1229 *dpp*-466. The *S. typhimurium* strains are derivatives of a motile LT2 strain²⁷: CH1104, *opp*-324 *tppB*16::Tn10; CH1110, CH1104 *dpp*-101::Tn5; CH1165, CH1104 *dpp*-152; CH1166, CH1104 *dpp*-150 (although phenotypically Dpp⁻, this last strain retains the periplasmic Dpp protein). Strain constructions and the use of the toxic peptides bacilysin and alafosfalin for the isolation and characterization of *dpp* mutants have been described elsewhere (refs 16, 20, 22 and M. M. Gibson and C.F.H., in preparation).

has a 49K protein associated with the dipeptide permease. The loss of this protein in only 15% of *dpp* mutants is compatible with the observation that binding protein-dependent transport systems require the function of a least four gene products^{32,33}. To demonstrate that this 49K periplasmic protein is required for peptide taxis, the *dpp* derivatives of RP437 were examined using the capillary assay. Taxis towards peptides, but not towards aspartate or serine, was abolished in those mutants that had lost the protein. Interestingly, one *dpp* mutant that had lost transport function but retained the 49K periplasmic protein still showed peptide taxis. Thus, the periplasmic protein itself, in the absence of transport activity, seems to serve as the primary receptor for peptide chemotaxis. This protein is the first example of a periplasmic chemotactic receptor for a substrate other than a sugar.

Peptide antibiotics are prevalent in nature. As the Dpp system, unlike the less fastidious Opp system, is specific and only tolerates minor modifications in peptide structure^{18,34}, use of the putative Dpp binding protein rather than OppA as the peptide chemoreceptor could prevent the cell from being attracted to potentially toxic compounds. The design of synthetic peptide antibiotics that can be recognized by Dpp may provide improved therapeutic effectiveness as the bacteria will be suicidally attracted towards the toxic moiety.

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Note added in proof: After submission of this manuscript Russo and Koshland³⁶ reported identification of a gene (*tip*) in *S. typhimurium* which might be analogous to *tap* in *E. coli*. While we did not detect peptide taxis in our *S. typhimurium* strains, we do not yet know whether they are *tip*⁺. Therefore, it remains possible that *tip* is equivalent to *tap* and serves to mediate dipeptide chemotaxis in *S. typhimurium*.

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Positively supercoiled DNA in a virus-like particle of an archaebacterium

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The topological state of DNA is of importance in a variety of essential biological events^{1–4}. Covalently closed circular DNA duplexes isolated from eukaryotic cells and their viruses, as well as from eubacteria and their bacteriophages are, without exception, negatively supercoiled. Little is known about the topological state of the DNA in archaebacteria, a group of organisms distinct from both eukaryotes and eubacteria⁵, but recently an ATP-dependent DNA topoisomerase has been isolated from the archaebacterium *Sulfolobus acidocaldarius*. This enzyme, termed 'reverse gyrase', converts relaxed or negatively supercoiled DNA into positively supercoiled forms *in vitro*^{6–9}. It is not a gyrase-like type II topoisomerase, as initially reported⁹, but an ATP-dependent type I enzyme^{7,8}. The function of this enzyme *in vivo* has not been

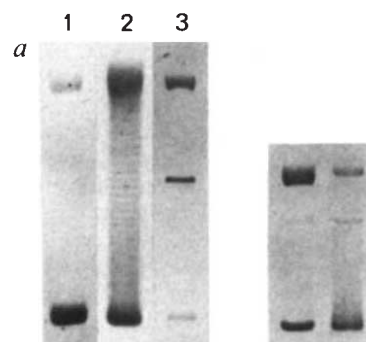


Fig. 1 Agarose gel electrophoresis of SSV-1 DNA. *a*, Electrophoresis in the absence of chloroquine. Lane 1, SSV-1 DNA extracted from the virus-like particle (referred to as 'viral' SSV-1). Lane 2, SSV-1 DNA extracted from the induced cell (referred to as 'cellular' SSV-1). Lane 3, cellular SSV-1 after partial digestion with the restriction endonuclease *Bam*HI. *b*, Chloroquine (20 µg ml⁻¹) was included in the gel and in the electrophoresis buffer. Lane 1, viral SSV-1. Lane 2, cellular SSV-1.

Methods. Conditions for growth of *Sulfolobus* strain B12 and ultraviolet (UV) induction of SSV-1 were essentially as described elsewhere¹⁰. After UV-irradiation of the culture, the pH was adjusted to 5.0 by adding dilute ammonia; 12 h after induction, cells were collected by low-speed centrifugation¹⁰. Purification of covalently closed circular DNA from induced cells was performed according to the protocol given in ref. 16. For CsCl₂/ethidium bromide density gradient centrifugation, however, *N*-lauroylsarcosine was added to 1% final concentration. The virus-like particle SSV-1 was purified from the culture supernatant essentially as described elsewhere¹⁰, but polyethylene glycol 6000 (10% final concentration) was used instead of ammonium sulphate. For purification of the viral DNA, a virus preparation was dialysed against 20 mM Tris-HCl pH 8.0, 1 mM EDTA; *N*-lauroylsarcosine was then added to 1% final concentration and the resulting solution was subjected to CsCl₂/ethidium bromide density gradient centrifugation. Under these conditions, a large proportion of the DNA (upper band in the gradient) appeared partially degraded, presumably owing to the culture conditions, while covalently closed circular DNA was found in the lower band. Electrophoresis was performed in 0.7% agarose gels for 15 h at 20°C and 1.7 V cm⁻¹ (normal gels) or 1 V cm⁻¹ (chloroquine gels). Gels were stained and photographed as described previously¹⁷. The electrophoresis buffer comprised 36 mM Tris, 30 mM NaH₂PO₄, 1 mM EDTA pH 7.8 (TEP buffer). Samples of SSV-1 DNA (0.3 µg) were subjected to electrophoresis. Resolution of individual topoisomers was incomplete owing to the large size of the DNA.

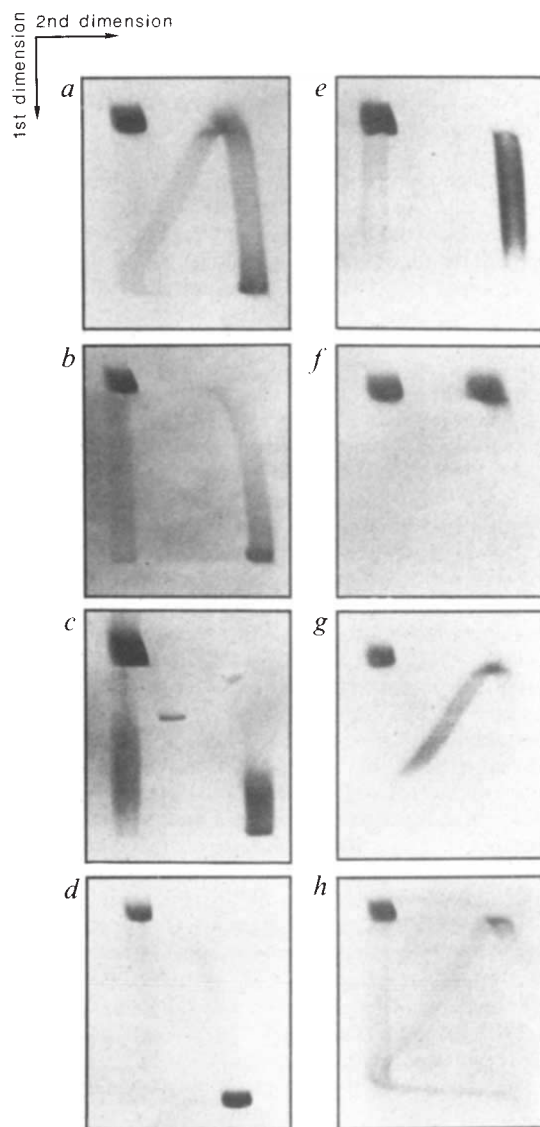
established, largely because of the lack of data on the topological state of *Sulfolobus* DNA. Here we show that DNA exists in a positively superhelical state in the genome of the virus-like particle SSV-1, present in *Sulfolobus*.

Sulfolobus isolate B12, an archaebacterium that grows at 80°C in acidic conditions, harbours an ultraviolet inducible virus-like particle¹⁰, now called SSV-1 (ref. 11). The genome of SSV-1 comprises 15.46 kilobases (kb) of DNA of known nucleotide sequence (P. Palm and B. Grampp, unpublished). This DNA is stably maintained in *Sulfolobus* B12 cells in two different forms. One copy of the DNA is site-specifically integrated into the genome; the other is present in the cells as a free plasmid with a low copy number¹². Although some of the lemon-shaped virus-like particles are released under normal culture conditions, by far the strongest stimulus for the production of SSV-1 is irradiation with a low dose of ultraviolet light, which results in the release of up to 5 × 10¹⁰ particles per ml (ref. 10). As yet, no other *Sulfolobus* strains infected with these particles have been demonstrated¹⁰. The SSV-1 DNA is actively transcribed under conditions of particle release and two genes coding for structural proteins of SSV-1 have been found on the plasmid DNA¹¹.

After exposure of *Sulfolobus* B12 cells to ultraviolet light, the DNA from SSV-1 was extracted using two different procedures.

Fig. 2 Analysis of SSV-1 DNAs in two-dimensional agarose gels. *a, d*, Untreated cellular and viral SSV-1 DNAs, respectively. *b*, Untreated cellular SSV-1 DNA in a gel containing $5 \mu\text{g ml}^{-1}$ chloroquine in the first dimension and $25 \mu\text{g ml}^{-1}$ chloroquine in the second dimension. *c*, Cellular SSV-1 after incubation with reverse gyrase. *e, f*, Viral SSV-1 after partial (*e*) or complete (*f*) relaxation by rat liver topoisomerase I. *g, h*, Viral SSV-1 made negatively supercoiled after incubation with topoisomerase I in the presence of $3.0 \mu\text{M}$ (*g*) or $10 \mu\text{M}$ (*h*) ethidium bromide. Note that some breaks occurred in the DNA during the incubation period between the first and the second dimension, producing an 'echo' below the position of form II. Linear DNA is visible in *c* and *e*.

Methods. Relaxation by rat liver topoisomerase I was performed in a $15\text{-}\mu\text{l}$ reaction mixture containing 10 mM Tris-HCl pH 7.9, 0.2 mM EDTA, 0.5 mM dithiothreitol (DTT), 0.2 M NaCl, $0.3 \mu\text{g}$ SSV-1 DNA and 8 units of topoisomerase I, purified according to ref. 18. Partial relaxation was obtained by incubation with 0.5 U of topoisomerase I. After 30 min at 37°C , the reaction was stopped by adding 1% SDS, 0.25 mg ml^{-1} bromophenol blue and 15% sucrose. Negative supercoils were produced under the same conditions with the following modifications: after initial incubation for 45 min at 37°C , $3.0\text{--}10.0 \mu\text{M}$ ethidium bromide and 8 U of topoisomerase I were added to the mixture and the incubation was continued for a further 45 min. Positive supercoiling by *S. acidocaldarius* reverse gyrase was performed in a $15\text{-}\mu\text{l}$ reaction mixture containing 50 mM Tris-HCl pH 7.9, 0.5 mM DTT, 10 mM MgCl_2 , 1 mM ATP, $30 \mu\text{g ml}^{-1}$ bovine serum albumin, $0.3 \mu\text{g}$ SSV-1 DNA and $2 \mu\text{l}$ of reverse gyrase (fraction III). Reverse gyrase was purified as described previously⁷. After 30 min at 75°C , the reaction was stopped as described above. Two-dimensional electrophoresis was performed essentially as described elsewhere¹³. In the first dimension, in the absence of chloroquine, the DNA migrated at 1.7 V cm^{-1} for 15 h at 20°C in TEP buffer. The gel was then soaked in TEP buffer containing $10 \mu\text{g ml}^{-1}$ chloroquine at room temperature for 8 h, after which the second dimension was run in the same buffer for 13 h at 1 V cm^{-1} . Chloroquine was eliminated by four incubations of 1 h each in distilled water. The gel was stained with $2 \mu\text{g ml}^{-1}$ ethidium bromide for 30 min and photographed as described previously¹⁷.



(1) Direct extraction from the induced cells yielded a DNA referred to as 'cellular' SSV-1 DNA. (2) Isolation of the viral particles, followed by extraction of the DNA, yielded 'viral' SSV-1 DNA (see Fig. 1 legend). In both cases, the DNA, purified by caesium chloride/ethidium bromide density gradient centrifugation, was a circular double-stranded (ds) DNA of 15.46 kb , which has been described previously¹². Incubation with exonuclease III confirmed that most of this DNA was covalently closed (not shown). In the virion preparation the DNA was found to be supercoiled as a typical form I (Fig. 1*a*, lane 1), while in the archaeobacterium (cellular SSV-1) its topological state ranged from completely relaxed to fully supercoiled (Fig. 1*a*, lane 2).

As shown previously¹², the restriction endonuclease *Bam*HI cuts SSV-1 DNA at only one site. Both of our DNA preparations yielded linear DNA which migrated between the positions typical of forms I and II (Fig. 1*a*, lane 3); this demonstrated the unique size of the plasmid DNA and confirmed that the multiple bands observed on agarose gels (lane 2) represented topoisomers.

Chloroquine, like ethidium bromide, decreases the twist of the double helix and consequently changes its superhelicity from negative values to zero, and finally to positive values¹³. Figure 1*b* shows an analysis of SSV-1 DNA in an electrophoresis gel containing $20 \mu\text{g ml}^{-1}$ chloroquine: both cellular (lane 1) and viral (lane 2) SSV-1 DNAs were fully supercoiled in these

conditions. This observation indicated that all the intermediate topoisomers of cellular SSV-1 (Fig. 1*a*, lane 2) were sensitive to chloroquine and became completely supercoiled within the gel. In contrast, the viral DNA and the highly supercoiled form of cellular DNA were apparently unaffected by such a chloroquine concentration, as well as by lower concentrations. This apparent discrepancy with regard to the intercalating effect of the dye suggests three different explanations for the structure of the highly supercoiled forms. These forms (1) had a high negative superhelical density, as in the case of plasmid pBR322 isolated from *supX* mutants, defective in type I topoisomerase¹⁴; (2) contained chemical or structural modifications; or (3) had a high positive superhelical density correlated with the existence of reverse gyration in *Sulfolobus* cells.

The positive superhelical state of the SSV-1 DNA was clearly demonstrated on two-dimensional agarose gels¹³ (Fig. 2). In addition, this procedure allowed the separation of the nicked circular form of SSV-1 largely present in all preparations. Figure 2*a* shows the two-dimensional gel pattern obtained for the cellular SSV-1 DNA. The resulting 'arch' of topoisomers displayed a wide distribution, quite unusual for a 'natural' DNA. The right-hand part of the arch, which represented positively supercoiled topoisomers¹³, was observed for the first time in a gel which did not contain chloroquine in the first dimension, and was largely predominant compared with the left-hand part of the arch (negative topoisomers). In this two-dimensional gel,

the most negative topoisomers migrated close to the major band of positive topoisomers; that is, the two extremities of the arch were close to one another (Fig. 2a). A clear separation of these topoisomers was achieved by introducing chloroquine in the first dimension (Fig. 2b): in these conditions, negative topoisomers were partly relaxed in the first dimension while positive topoisomers were made more supercoiled, so that the whole distribution was shifted to the right of the arch (Fig. 2b). Furthermore, instead of using chloroquine in the first dimension, the intermediate topoisomers of cellular SSV-1 DNA can be made more supercoiled *in vitro* by incubation with *S. acidocaldarius* reverse gyrase (Fig. 2c), an enzyme that creates (but does not remove) positive supercoils⁷. This reaction, which uses ATP, has been described previously with pBR322 as a substrate⁷; these results strongly suggested that the major band of cellular SSV-1 visible at the right end of the arch in Fig. 2a-c comprised highly positive topoisomers. In contrast to cellular DNA, the viral SSV-1 DNA appeared fully positively supercoiled (Fig. 2d) and the addition of chloroquine to the gel or treatment with reverse gyrase had no effect (not shown).

Other lines of evidence for positive supercoiling include the following. (1) Both DNAs can be relaxed by the action of a eukaryotic type I topoisomerase which relaxes positive and negative supercoils by introducing transient single-strand breaks into the DNA (Fig. 2f): SSV-1 DNAs are not knotted forms, as knots are only removed by a type II topoisomerase. (2) Partial relaxation of viral SSV-1 DNA produces a set of topoisomers that are positively supercoiled when analysed in a two-dimensional gel (Fig. 2e). (3) SSV-1 DNAs are not relaxed by incubation with *Escherichia coli* topoisomerase I (protein ω), which only relaxes negative supercoils (not shown).

Yet another line of evidence derives from the comparison of negatively supercoiled forms of SSV-1 DNA prepared *in vitro* with natural SSV-1 DNAs. Indeed, it is possible to convert cellular or viral SSV-1 DNAs to negatively supercoiled DNA by incubation with eukaryotic type I topoisomerase in the presence of ethidium bromide; Fig. 2g, h shows two such DNAs with different superhelical densities. In contrast to natural SSV-1 DNAs (Fig. 2a, d), the migration of these negatively supercoiled DNAs is reduced in a chloroquine gel.

The experiments described here provide strong evidence for the existence of positively supercoiled DNA *in vivo*. Indeed, we have shown previously that high positive supercoiling can also be achieved *in vitro* by using the reverse gyrase of *Sulfolobus* in the presence of polyethylene glycol (PEG)⁷. PEG increases the local concentration of the reactants, providing conditions presumably similar to those existing *in vivo*. Positive supercoiling of SSV-1 DNA could alternatively be explained by assuming a protein-DNA interaction within the cells that leads to a negatively supercoiled nucleoprotein. After the relaxation of such a complex by a topoisomerase, the removal of the protein *in vitro* would give rise to a positively supercoiled molecule. This possibility seems unlikely as efficient supercoiling *in vitro* can be achieved using the reverse gyrase alone in clearly catalytic conditions⁷.

The observation of the two forms of SSV-1 DNA, one fully supercoiled in the virion, the other found in the bacterium as a wide spectrum of topoisomers (including a small proportion of negative topoisomers), is intriguing. One possible explanation is that within the bacterium, the viral DNA (positively supercoiled) is made negatively supercoiled during transcription and replication, then condensed into a highly positive supercoiled form for encapsidation. The existence of negatively supercoiled and partially relaxed SSV-1 DNA in the bacterium suggests that another topoisomerase, an antagonist of reverse gyrase, is present. An enzyme that can relax positively supercoiled DNA has been identified recently in *S. acidocaldarius*⁸; it is not clear, however, whether this enzyme is a type II topoisomerase analogous to phage T₄ enzyme, or whether its activity is similar to that of eubacterial gyrase. These different observations raise

further questions: is the genome of *Sulfolobus* itself positively supercoiled (presumably by the activity of reverse gyrase)? Do the variations in the topological state of SSV-1 DNA reflect any topological regulation of the *Sulfolobus* genome?

The role of reverse gyrase presumably is not restricted to the packaging of viral DNA, as reverse gyrase is abundant in uninfected *Sulfolobus* strains. Thus, one can hypothesize that the genome (or at least some domains) of *Sulfolobus* itself is positively supercoiled *in vivo* and that the level of superhelical density is regulated. Positive supercoiling might be required both to stabilize the DNA in high-temperature (80 °C) growth conditions (to prevent uncontrolled gene expression at this temperature) and to inactivate the genes.

It remains to be determined whether positively supercoiled DNA is restricted to SSV-1 and/or to the genus *Sulfolobus*, or whether it also exists in other groups of archaebacteria, eubacteria and perhaps in some eukaryotes. Recently, a nuclear factor from myeloma cells has been shown to promote positive supercoiling *in vitro*¹⁵. As the eukaryotic genome is organized in topologically independent chromatin domains, an attractive hypothesis is that relaxation, or even positive supercoiling, of some of these domains would result in an inactivation of the corresponding genes; this may constitute another mode of gene regulation and expression.

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Corrigendum

Specific growth response of *ras*-transformed embryo fibroblasts to tumour promoters

G. P. Dotto, L. F. Parada & R. A. Weinberg
Nature **318**, 472-475 (1985)

THIS letter reported that a phorbol ester tumour promoter acted synergistically with an activated *ras* oncogene to stimulate the outgrowth of transformed foci in early passage rat embryo fibroblast cell cultures. However, the authors neglected to cite an earlier study by Hsiao, W., Gattioni-Celli, S. and Weinstein, I. B. (*Science* **226**, 552-555; 1984) which described a similar phenomenon using the C3H 10T1/2 mouse embryo fibroblast cell line.

Erratum

The last pluvial climatic episodes in the deserts of southwestern North America

W. G. Spaulding & L. J. Graumlich *Nature* **320**, 441-444 (1986)

IN the second paragraph on page 444, line 4 should read "... and the Grand Canyon³⁴, ...". This refers to K. L. Cole's work in ref. 34. In addition, the cover caption to this issue (3 April) moved the site shown (Moctezuma Head 1) to southern California. It is in fact in the Ajo Mountains of southern Arizona.